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NOTES ON HELMINTHS FROM PANAMA¹

I. *TAXORCHIS SCHISTOCOTYLE* (FISCHÖEDER, 1901), FROM
THE PANAMANIAN CAPYBARA, *HYDROCHOERUS*
ISTHMIUS GOLDMAN, 1912

ERNEST CARROLL FAUST

INTRODUCTION

Among three adult and three half-grown capybaras obtained during the month of August, 1930, in Darien Province, Republic of Panama, one large young male, weighing 46½ pounds, was autopsied almost immediately after the animal had died of gunshot wounds. On opening the abdomen twenty-nine healthy specimens of amphistomate flukes were found in the peritoneal cavity. Some of these were actively crawling out of the gunshot wounds. Others were so firmly anchored to the peritoneal wall by their posterior suckers that they were pulled in two by forceps traction. Careful inspection failed to reveal any lesion of the intestinal tract through which they might have issued, and examination of the intestine failed to discover any additional specimens of the fluke in that locality. It was with some reluctance, therefore, that the worms were recorded as parasites of the peritoneal cavity.

DESCRIPTION OF THE FLUKE

Opportunity was afforded to study the flukes carefully in the living condition. They were a clear pinkish flesh color, more or less ovate in contour, when viewed from the ventral side (Fig. 1, a), and on lateral aspect looked more like a truncated pyramid having rounded corners with the base at the posterior extremity (Fig. 1, b). They were very active and in physiologic saline solution changed form continuously. It was difficult to obtain accurate measurements of the living worms; they averaged about 12.5 mm. in length by 7.5 mm. in greatest breadth (at the anterior plane of the posterior sucker). With the unaided eye it was easy to recognize the subterminal oral sucker, the much smaller

¹ Contribution from the Parasitology Laboratory, Department of Tropical Medicine, Tulane University, New Orleans, La., and from the Gorgas Memorial Laboratory, Republic of Panama. The material which forms the basis of this study was obtained during the summer of 1930, when the writer was a guest of Dr. H. C. Clark, Director of the Gorgas Memorial Laboratory.

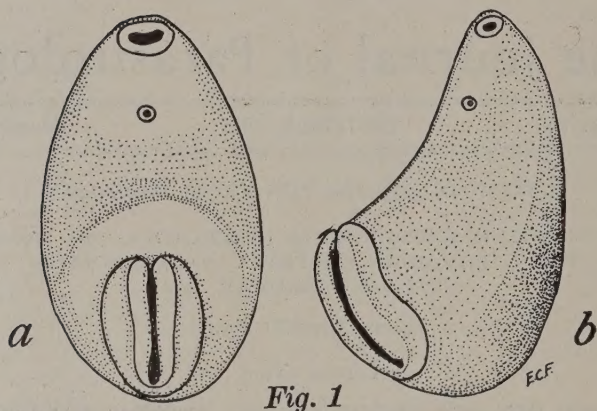


Fig. 1

Fig. 1. Habit sketches of *Taxorchis schistocotyle* in uncompressed condition; a, ventral view, b, lateral view. $\times 3$.

genital sucker in the mid-line a short distance behind the oral sucker, and the large pyriform posterior sucker, which had a "slit" or fissure at its posteroventral border. On compression between two microscopic slides, the worms became more broadly ovate, with the greatest diameter just behind the level of the genital sucker. The integument was aspinose. They were relatively transparent and it was possible to make out the shape and position of the important landmarks. Later, while the flukes were still alive, 23 specimens were fixed in steaming Schaudinn's fluid, eleven compressed between slides and twelve in the uncompressed condition. Some of the compressed fixed specimens were stained and mounted as *toto* objects. Several of the uncompressed fixed worms were sacrificed in attempts to obtain serial sections, but great difficulty was experienced in infiltrating them with paraffin. Finally two specimens were adequately infiltrated with 56° C. paraffin and provided almost perfect series of frontal and transverse sections of 10 microns thickness.

Data have been assembled to demonstrate that the flukes belong to the species *Taxorchis schistocotyle* (Fischöeder, 1901), which was first described from two museum specimens obtained from the Brazilian peccary, *Dicotyles torquatus*, and later obtained by Travassos (1923, 1925, 1927) from the large intestine of *Hydrochoerus capibara* in Brazil. Originally described as a subgenus, Maplestone (1923) elevated *Taxorchis* to generic rank. However, the present writer is not able to find any morphological description of *Taxorchis schistocotyle* other than that provided by Fischöeder, who, because he possessed only two specimens for study, did not feel it was wise to section either one. His descrip-

tion, therefore, is limited to *toto* mounts, one with a ventral aspect uppermost and one in lateral view. Both of these worms were considerably longer (20.5 and 19.5 mm.) than the writer's material, although the transverse measurements were approximately the same (7.0 and 8.5 mm.). In spite of Fiscoeder's handicap in having only preserved material and no serial sections on which to work, his descriptions are exceedingly accurate, although not as detailed as his data on related forms, in which he was able to reconstruct the genital organs from sections.

In the partly compressed worm (Fig. 2) the various organs are

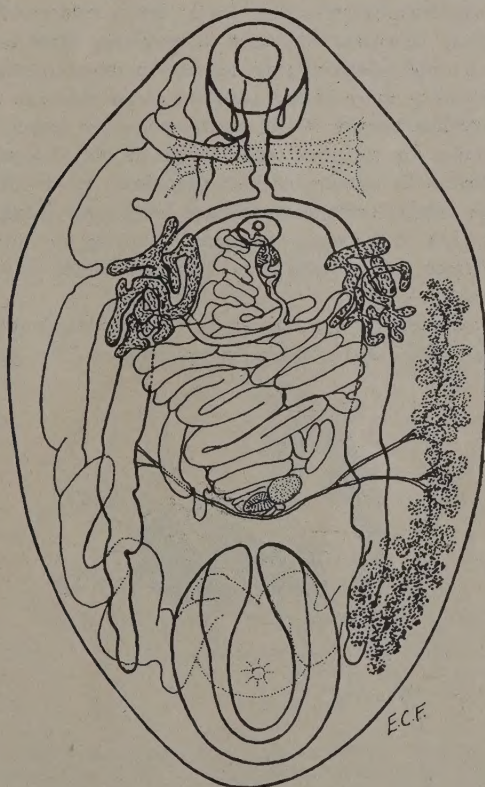


Fig. 2

Fig. 2. Ventral view of a compressed specimen of living *T. schistocotyle*, showing important organs, including the characteristic outline and position of the testes. The vitellaria are shown only on the left of the animal, the excretory system only on the right. Note the characteristic fissure of the posterior sucker. Camera lucida, $\times 6$.

situated in essentially the same relative positions as in uncompressed material. In addition to the conspicuous suckers, both living and preserved specimens show the wish-bone type of intestine, with slightly serpentine ceca, which extend posteriad to the mid-plane of the posterior sucker. The paired testes, with long, finger-like, curved branches, occupy a position immediately ventral to the ceca, at the level just posterior to the genital sucker. The ovary lies slightly to the side of the mid-sagittal plane, while the main mass of the uterus is confined mesad to the intestinal ceca, between the ovary and the genital sucker. The vitelline glands lie in the lateral fields, almost completely outside the intestinal ceca, extending from the level of the testes to the posterior end of the ceca. They are composed of numerous large oval to polygonally-shaped units, which appear discrete in *toto* mounts but in sections are seen to be more or less fused or connected with one another. The excretory bladder lies dorsal to the posterior sucker. The main collecting tubules are tortuous canals which lie dorsal to the vitellaria. They are filled with minute blackish granules. In the region of the pharynx these tubules become constricted, bend inward and then downward and are lost in the mass of organs occupying the intercecal field. Attempts to trace the secondary and tertiary tubules in sections have been unsuccessful.

Detailed examination of serial sections was undertaken to determine the exact relationship of the female genital complex (Fig. 3). The sub-

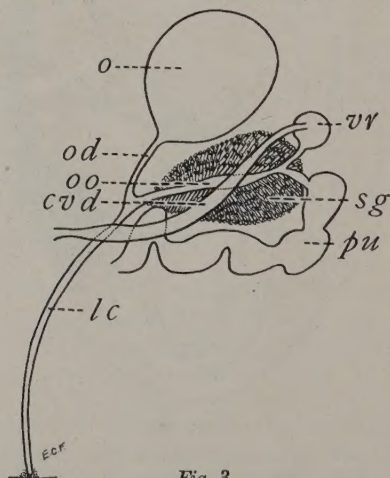


Fig. 3

Fig. 3. Semidiagrammatic sketch, based on camera lucida drawings of serial sections, of the oötype and adjacent genital organs. *cvd*, common vitelline duct; *l. c.*, Laurer's canal; *o*, ovary; *od*, oviduct, *oo*, oötype; *pu*, proximal end of uterus; *sg*, "shell gland"; *vr*, vitelline reservoir. $\times$ ca 30.

spherical ovary (*o*), of small size, lies to the right of the mid-sagittal plane on the ventral side of the fluke. A short delicate oviduct (*od*) leads posteriad and slightly mesad. It is joined by a long delicate Laurer's canal (*lc*), which is open along its entire length but ends blindly just under the dorsal integument. It contained no spermatozoa in the specimens studied. There is no seminal receptacle. The oötype (*oo*) with its surrounding gland is pyriform; its median end is the smaller. It lies just posterior to the ovary. From its median aspect it receives the common duct formed by the oviduct and Laurer's canal. The gland surrounding the oötype (*sg*) consists of small multiple units. At its outer end are two ducts. One is the distal terminus of the common vitelline duct (*cvd*), with its small spherical vitelline reservoir (*vr*). The other is the inner end of the uterus (*pu*), which soon expands to a diameter large enough to accommodate the fertilized eggs and great masses of spermatozoa. These organs constitute the inner female genitalia.

Equal care and a considerably greater amount of time was necessary to reconstruct from sections the male and female organs in the vicinity

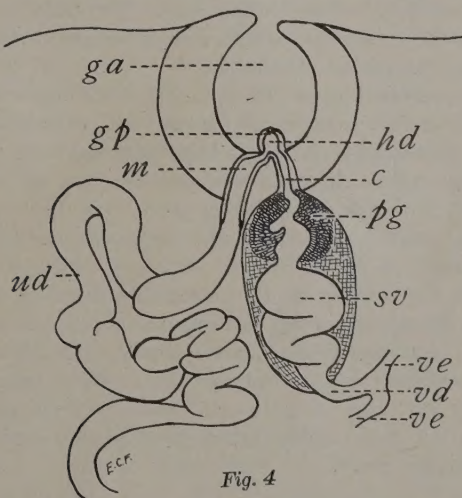


Fig. 4

Fig. 4. Semidiagrammatic sketch based on camera lucida drawings of serial sections, of the genitalia in the immediate vicinity of the genital sucker. The dorsal aspect is on the right, the ventral aspect, on the left. *c*, cirrus tube; *ga*, genital atrium; *gp*, genital pore; *hd*, hermaphroditic duct; *m*, metraterm; *pg*, prostate gland; *sv*, seminal vesicle; *ud*, distal end of uterus; *vd*, vas deferens; *ve*, vas efferens. $\times$ ca 30.

of the genital sucker (Fig. 4). Ventral to the transverse mid-plane of the genital sucker the outer coils of the uterus (*ud*) wind a tortuous

course toward the genital atrium. Just before entering the lower rim of the sucker, the uterine wall becomes thicker and more muscular (*m*). This thickened portion (the metraterm) continues through the sucker wall and merges with the more dorsally situated male duct to form the hermaphroditic duct (*hd*). The outer portion of the male system arises as two relatively thin-walled, large-calibered vasa efferentia (*ve*) from the more anterior portion of the main stems of the paired right and left testes. These right and left tubules, on arriving near the mid-sagittal plane, join to form the short vas deferens (*vd*), which almost immediately enters the cirrus sac. This sac is a bulbous organ and consists of two parts, a posterior region enclosing the tightly coiled seminal vesicle (*sv*) and an anterior coiled portion surrounded by the mass of the prostrate gland (*pg*). The wall of the cirrus sac is somewhat muscular. Between it and the enclosed seminal vesicle is a meshwork of rather dense fibrous parenchymatous tissue. Continuing ventrad from the cirrus sac through the well of the genital sucker there is a short, small-calibered, muscular tubule, which is the cirrus tube (*c*); there is no evidence of a protrusible penial organ. This tube joins the metraterm to form the short hermaphroditic duct. This latter duct is situated in a small domed prominence, which projects into the cavity of the genital sucker (*i.e.*, the genital atrium, *ga*).

From his preserved (and probably shrunken) material Fiscoeder (1903) gave the measurements of the eggs of *Taxorchis schistocotyle* as 145–156 μ in length by 72–80 μ in transverse diameter. The writer's living specimens (from the outer uterine coils) had egg measurements ranging from 174 to 214 μ in length by 90 to 118 μ in breadth, with an average of $204 \times 105 \mu$. Preserved specimens contained eggs somewhat smaller. Because of the differences in the condition of the material examined and the absence of other essentially different morphological features, this discrepancy in egg size can not be regarded as specifically significant. These eggs (Fig. 5) are typically ovate, have a small but prominent operculum and a relatively thin, tough shell, and contain embryos from the stage of early cell cleavage to the gastrula stage. Without question they require further development outside the worm before hatching can occur.

THE NORMAL HABITS OF TAXORCHIS

The discovery of this colony of *Taxorchis schistocotyle* only in the peritoneal cavity of their host, without any apparent opening of the large bowel wall suggesting migration from the more normal habitat of these worms, has raised the question of how and when they arrived in the body cavity. It is known that *Paragonimus westermani*, which as a metacercarial larva migrates from the intestinal lumen through the

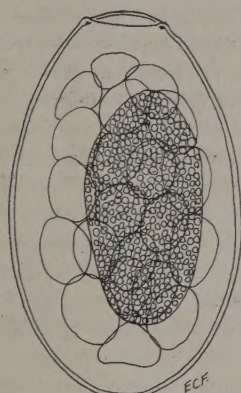


Fig. 5

Fig. 5. Camera lucida sketch of egg of *T. schistocotyle* about to be laid by living worm. Note epibolic development of the embryo. $\times 315$.

bowel wall, at times matures in the peritoneal or other body cavities, but is invariably surrounded by an adventitious capsule and is never as an adult unconfined in the cavity. In spite of the apparent absence of trauma or lesions in the bowel wall of the parasitized capybara, the sections of the worms provided evidence that they had quite recently been in the host's intestinal tract, since their ceca contained numerous specimens of ciliates, particularly species of the genus *Cycloposthium* Bundle, 1895, which Da Cunha (1915) described from the Brazilian capybara and which the writer commonly found in the cecum of all Panamanian capybaras examined. It is evident, therefore, that these worms normally inhabited the cecum of the particular host under consideration, and that the flukes either of their own accord worked their way through undamaged tissue of the intestinal wall, or, more likely, migrated through lesions which were not discovered at the time of autopsy.

As has been previously suggested (Faust, 1931), it is possible that this is the same species of fluke which Dr. Clark collected from the cecum of the wild hog in the Coto region of Northern Panama in February (1929).

POSSIBLE CLUES TO THE LIFE CYCLE

In line with the life cycles of other paramphistomate flukes, the development of *Taxorchis schistocotyle* should logically include the following stages: (1) the eggs, passed in the feces of the capybara while in swampy ground, maturing in the water, and hatching, so that the

miracidia may penetrate the appropriate molluscan host; (2) within the mollusc the miracidia metamorphosing into sporocysts, which produce rediae, the latter, in turn, producing daughter rediae and/or cercariae; (3) the cercariae emerging from the mollusc, swimming about through the water, crawling upon water grass and encysting; (4) the infected grass, consumed by herbivorous mammals, with excystation of the metacercariae occurring in the small intestine of the host and the young flukes passing down to the cecum, where they mature and lay their eggs. Following this probable course of events, attempts were made to find the intermediate stages in species of molluscs living in the Boco de Cupe swamps where the capybaras were commonly found. Ten large specimens of one species of snail, *Ampullaria cumingii* King, 1850-51, kindly identified by Mr. James Zetek, were collected. On examination one specimen was found to contain several mother rediae (second generation parthenitae) and a few daughter rediae (third generation parthenitae). No cercariae were seen, probably because of the early stage in the development of the intramolluscan phase of the life cycle. Most interesting

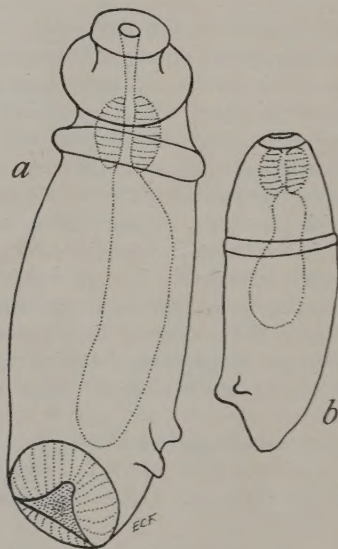


Fig. 6

Fig. 6. Rediae of amphistomate (?) fluke from the interglandular lymph spaces of *Ampullaria cumingii*, from the same habitat from which the host of *T. schistocotyle* was taken. *a*, mother redia; *b*, daughter redia. Note the unique aboral sucker, and the peculiar snout-like oral sucker of the mother redia. $\times 18$.

and possibly significant was the structure of the mother redia (Fig. 6, *a*), which had a large snout-shaped oral sucker and at the aboral end a slightly smaller blind acetabulum. In contrast (Fig. 6, *b*) the daughter redia was specifically undifferentiable from the rediae of many amphistomate and distomate species. Both parent and daughter rediae had small but distinct locomotor appendages. The extraordinary structure in the mother rediae was the aboral acetabulum, which the literature on the subject fails to describe or figure.

While the evidence connecting these redial stages of a digenetic trematode in the molluscan host, *Ampullaria cumingii* with *Taxorchis schistocotyle* in the caypara is quite inadequate, and while there is even no proof that the rediae are paramphistomate in type, it has seemed desirable to put these findings on record as possibly suggestive of the intermediate phase of the life cycle of this amphistome.

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NOTES ON HELMINTHS FROM PANAMA¹

II. RARE HUMAN NEMATODE EGGS IN THE FECES OF INDIVIDUALS FROM THE CHAGRES RIVER, PANAMA

ERNEST CARROLL FAUST AND WILLIAM H. MARTINEZ

In a preliminary communication (Faust, 1931) brief mention was made of the finding of *Capillaria (vel Hepaticola) hepatica* and *Gongylonema* eggs during intensive routine fecal examinations of the human population of the Chagres River basin, Republic of Panama, in 1930. During this survey practically all of the individuals in five small, closely associated villages were subjected to three consecutive fecal examinations. Altogether 440 persons were included in the series. The population is negroid, having mostly migrated from Jamaica and settled in the area a generation or so ago. In addition to a high infective parasite index (3.0), including 31.8 per cent infection with *Endamoeba histolytica*, 42.9 per cent with *Ascaris*, 73.4 per cent with *Necator*, 44.3 per cent with *Trichocephalus* and 19.5 per cent with *Strongyloides stercoralis*, certain members of the group passed eggs in their feces which have rarely been recorded from man.

CAPILLARIA HEPATICA EGGS

In nine individuals, three from one village (Las Vegas), two each from Guabalito and Gatuncillo, and one each from Santa Rosa and Las Huecas, the original fresh fecal specimens contained typical eggs of *Capillaria hepatica* (*vide* Baylis, 1931). Interestingly enough all of these persons were related by birth or marriage and frequently visited one another. In two of the cases the eggs were relatively common; in six they were relatively few; in one they were found only after centrifugation of the specimen. It was realized that while this parasite is quite common as an hepatic infection in rats, only one authentic human case was on record, also a liver involvement (MacArthur, 1924). The question therefore arose as to how the eggs of this worm might be discharged in human feces. Subsequently fecal examination was negative for seven of the nine individuals, but in two the eggs were recovered plentifully in both gross films and concentrates. Sputum and urine of all nine were consistently negative for the eggs.

It was determined to bring one consistent "egg passer" (a young married female) to the Santo Tomas Hospital in Panama City for ob-

¹ Contribution from the Parasitology Laboratory, Department of Tropical Medicine, Tulane University, New Orleans, La., and from the Gorgas Memorial Laboratory, Republic of Panama. The material which forms the basis of this study was obtained during the summer of 1930, when the writers were guests of Dr. H. C. Clark, Director of the Gorgas Memorial Laboratory.

servation and possible treatment. On the day of admission the stool was still positive for *Capillaria hepatica* eggs. After purgation with magnesium sulphate only hookworm and *Ascaris* eggs were found. Administration of 3 cc. tetrachlorethylene resulted in the passage of 46 necators and 1 minute *Ascaris*. Subsequently 2 ounces of fresh leche de higueron produced no further discharge of worms. Examination of fluid recovered through a duodenal tube was also negative. During a subsequent ten-day period in the hospital the stools remained negative for helminth eggs. It was concluded, therefore, that this patient, and probably the other cases, were not actually parasitized by the worm, *Capillaria hepatica*, but that the eggs passed in the feces had been ingested as contaminations and discharged in an essentially unaltered condition through the intestinal tract of these individuals.

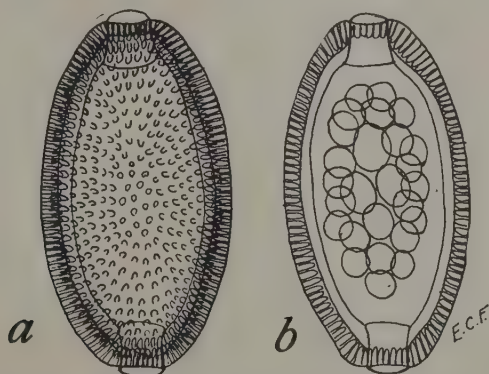


Fig. 1. Eggs of *Capillaria hepatica* from human feces, Chagres River, Panama. *a*, surface view, showing symmetrical channeling; *b*, optional view, with morula-stage embryo inside. Camera lucida, $\times 1000$.

The source of the contamination was the next subject of inquiry. The persons who passed the eggs vigorously denied that they ever ate rats, uncooked, or cooked, but admitted that rats commonly infested their huts. No other animals were kept in the homes of these families except dogs and cats. There remained three possible explanations: (1) rats parasitized with *C. hepatica* had died and decayed in or near their homes and had contaminated food or drink of the individuals; (2) dogs or cats had eaten parasitized rats and the feces of these animals had contaminated food or drink of the "positive" persons; or (3) the persons who passed the eggs had eaten livers of dogs infected with *C. hepatica* (Wright, 1930). The latter two hypotheses seem the more likely explanation. Unfortunately opportunity to test these hypotheses by autopsy of village dogs and cats was refused. Since the preliminary

publication, (*l.c.*) Vogel (1932), has recorded the presence of *C. hepatica* eggs in the feces of a young male in French Guinea. This investigator believed that the eggs were merely *en transit* through the human intestine and had been derived from an infection in the livers of wild rats, which he found commonly to harbor the parasite in that area. Likewise, Sandground (1933) has reported the passage of this type of egg in the feces of a native woman at Mt. Silinda, Southern Rhodesia, in which vicinity field mice are usually heavily infected with the worm.

Most of the eggs seen by us were in the blastula stage (Fig. 1, *a, b*), although a few were as immature as the two-cell stage. They measured

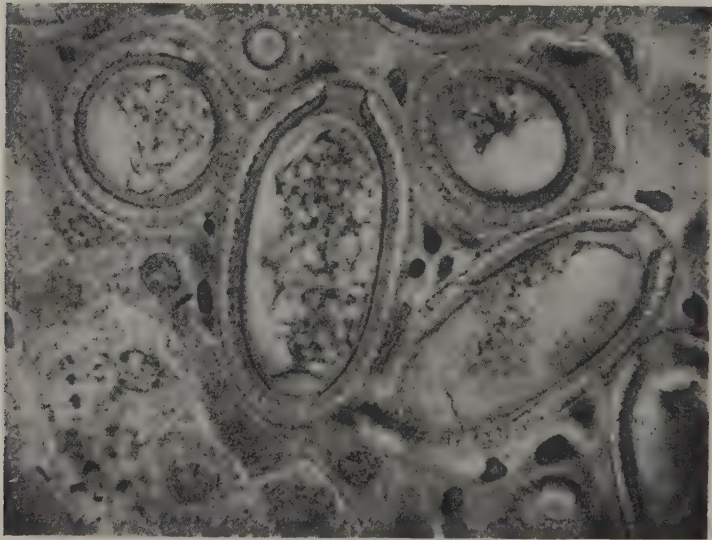


Fig. 2. Photomicrograph of *Capillaria hepatica* in liver section of infected rat. $\times 1000$. (Original.)

55–59 μ by 27–30 μ . The majority appeared to be viable, but attempt to induce further embryonation by culturing them on moist sand, moist charcoal or moist blotting paper, with and without weak formalin solution, were all negative. That these eggs were actually those of *Capillaria hepatica* can be readily determined by comparison of the camera lucida sketches (Fig. 1, *a, b*) with a photomicrograph of eggs of this species in a section of a rat's liver (Fig. 2). On the basis of Baylis' description (1926) of the egg of the related species, *C. gastrica*, which lacks the striated or channeled sculpturing of the outer shell so characteristic of *C. hepatica* eggs, the eggs found by us cannot be confused

with those of species inhabiting the stomach. It is clear, however, that the Chagres River "infections" were not actual infections but were merely contaminations, and that the only authentic record of human infection by this nematode is the one reported by MacArthur (*l.c.*).

GONGYLONEMA EGGS

In one adult male, resident of the village of Gatuncillo, examined during the 1930 survey, eggs identical with those of *Gongylonema pulchrum* were recovered from the feces once out of three examinations. These eggs (Fig. 3) measured $51.2 \times 27.2 \mu$, were light lemon-colored,

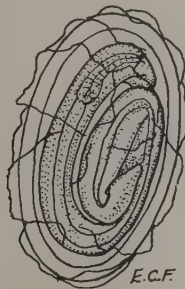


Fig. 3. Egg of *Gongylonema* from human feces, Chagres River, Panama. Camera lucida, $\times 1000$.

had a thick, regular oval shell, which was quite refractive, and possessed a thin wrinkled outer membrane. Within the shell there was a tightly coiled, mature or nearly mature, rhabditiform larva. In view of the fact that the eggs were found in only one of three fecal examinations, and in the absence of any physical signs or clinical evidence of *Gongylonema* or other spiruroid infection in the individual examined, it is logical to believe that this was probably not a human infection but a contamination of the digestive tract.

Supporting this view is the following epidemiological evidence. The village in which the man lived was the only one in the Chagres River group where pigs were tolerated. The house in which he lived was built over a pigsty and he himself tended the pigs. Feces of these pigs contained eggs identical in character to those recovered from the man. Cockroaches were very abundant around the house and might be easily eaten as food. While the person examined was probably not actually infected, as indicated by the evidence, conditions in the immediate vicinity were favorable for human infection.

COMMENT

The more recent helminthological literature, particularly that from Asia, has indicated the relatively common confusion between true and

false helminthiases, especially distomiasis, including data on *Fasciola* and *Dicrocoelium* eggs in human stools acquired from consumption of infected sheep and goat livers (Strom, 1927, Curran and Feng, 1930, Tarasoff, 1932, *et al.*), as well as the eggs of a fish trematode in human stools after consumption of infected fish (Tubangui and Francisco, 1930). In pseudo-infections, where the diet of the patient was controlled, the eggs of the fluke ceased to appear. One of us (E. C. F.) once found a spiruroid egg in the feces of a Chinese medical student; repeated subsequent examinations proved negative. Likewise *Ascaris lumbricoides* eggs are not uncommonly found in the feces of coprophagous dogs. In order that the literature may not be burdened with pseudo-helminth records inseparable from actual infections, it is important to check each suspected case and to determine its real status.

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THE MOTHER SPORO CYSTS OF *LEUCOCHLORIDIUM*¹

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The two outstanding groups of Trematodes whose branching sporocysts have often been described are the Gasterostomes and Harmostomes. In the latter group most of the work has been done on *Leucochloridium*. In a recent paper (Woodhead, 1931) I described the redia of the Gasterostomes and showed how it remained a life-long captive within the lumen of the mother-sporocyst. This finding so stimulated my interest that when a source of *Leucochloridium* was called to my attention, I seized the opportunity to secure specimens and search for the redia or daughter sporocyst which I felt should exist. Furthermore it seemed to me that *Leucochloridium* might furnish good material for further investigation into the subject of spermatogenesis in sporocysts and rediae.

McIntosh (1932) has given us a timely paper on adult *Leucochloridium* secured from many species of birds. It appears from his work that *Leucochloridium* is quite specific as to its final host and that for each species a different form of sporocyst should be found in snails. In my work of the last three years on *Succinia retusa* it soon became evident that several forms of sporocysts were being dissected from the snails although only one color of mature sporocyst was recognized. The low water level of Lake Erie during the spring of 1934 permitted the gathering of about twenty thousand snails with comparative ease. Examination resulted in the finding of three new colored sporocysts in addition to the red-brown known for the last few years. It was deemed advisable to describe the sporocysts found and to reserve all mention of the peculiar histology and the method of reproduction for a later paper.

MATERIALS

In the latter part of May, 1930, a single dead specimen of *Succinia retusa* parasitized with *Leucochloridium* was given to me by a collector from the Museum of Zoology. Early on the morning of May 7, 1931, a collection was made in the same area covered by the collector of the previous year, namely; the marshes on the west shore of Lake Erie about 8 miles north of Toledo, Ohio. The results of this collecting and the many succeeding are summarized in table 1.

The first sight of the "pulsating brood-sacs" in the tentacles of the snails is a thrilling sight to a parasitologist. On the first collecting trip, a brood-sac was found that had broken out of the tentacle and, still

¹ Contributions from the Zoological Laboratory of the University of Michigan.

TABLE I.—*Collection Data*

Date	Locality	No. snails	Sporocyst	Weather	Remarks
5- 7-1930	Erie Marsh	1	1 Red-brown	Moist-clear-cool	None dissected
5- 7-1931	"	800	50 "	Dry " warm	50 young stages
9-15-1931	"	200	10 "	Dry " "	56 "
5- 6-1931	"	2000	4 "	Moist "	Vegetation flat
10-21-1932	"	400	15 "	Dry " cool	"
7- 3-1933	"	200	13 "	" " warm	high, heavy
5-26-1934	"	5000	220 " 15 green	Moist " cool	Mud-flats dry
6-10-1934	"	6000	350 " 36 "	" " "	No vegetation
7- 4-1934	"	5000	300 " 20 "	" " warm	Sagittaria 1 ft. high
7-13-1934	Guard Is.	50	5 " 2 "	Dry " very hot	10 a. m.—2 p. m.—Birds
7-13-1934	Erie Marsh	2000	100 " 16 "	Moist-cloudy-cool	Rain 4 p. m.
7-20-1934	"	6	2 " 2 "	Dry-clear-very hot	Club area
8- 4-1934	"	3000	7 " 5 "	" " warm	Vegetation heavy

attached by a thread-like process, was being dragged slowly over the ground by the snail. Stimulated no doubt by contact with the ground, the pulsating sac had all the appearance of a crawling maggot. This observation may indicate how the bird-host acquires the infection. For one-half hour a member of a collecting party watched a sporocyst that had emerged about two-thirds of the way from the snail. The latter remained motionless. Finally giving up hopes of the process being completed, the snail was picked up. Immediately the sporocyst dropped out completely. This confirms a previous field observation that the snail must aid the freeing of the brood-sac by crawling away. When water is forced into the inlet by an easterly wind, the marsh is covered to a depth of from 1 to 6 inches. The snails are forced either to the margin among the cat-tails or to ascend the stems of the plants growing out of water. By the middle of July there was a noticeable decrease in the number of parasitized snails which we attributed not alone to our many visits but also to the fact that the high peak of shedding of brood-sacs had been passed. The mud area now showed an enormous number of newly hatched snails and many empty shells of the 2-3 year age group. All snails not showing active sporocysts were planted in likely areas near Ann Arbor, in charge of a graduate student.

TECHNIQUE

For the purpose of experiment the following fixatives were used: Carnoy's No. 2, Bouin's, Zenker's, sublimate-acetic, Gilson's and formalin. Bouin's fluid gave by far the best results. Many specimens were laboriously dissected free from liver tissue before fixation. A few of these were sectioned and the remainder stained and mounted *in toto*. It was found that both Gilson's fluid and 5 per cent formalin dissolved the green color in the green sporocysts while Bouin's fluid did not dissolve it. Entire snails were narcotized with menthol and then dropped into hot formol-corrosive. By this method brood-sacs showed in the neck and tentacles of the extended snail.

OBSERVATIONS

The Red-Brown Sporocyst: Of the four differently colored sporocysts described in this paper, the red-browns are by far the most common. The month of June is indicated as the best collecting time for visible brood-sacs. Late September has proven in the past to be the best season for young developing sporocysts, as the infection apparently is acquired by young snails during the summer. The parasite develops during the fall and winter, producing metacercaria in time to infect young birds during the next spring nesting season. Mature sacs, however, may be gathered in the field during the entire summer and early fall from older snails.

In my specimens the crown is smoothly pigmented, not spotted or mottled. This crown is colored red-brown. Immediately below the crown is a narrow ring of creamy-white followed by a broad band of darker red-brown. Then a broad band of creamy-white succeeded by a broad area of red-brown interspersed with two irregular, not clean-cut, rings of creamy-white. In most specimens these white rings are connected across the red-brown band by narrow strips of white. Below these rings is a broad area of creamy-yellow and then a broad band of dispersed pigment, a red-brown on the margins and a deep brown mottled ring in the center of the band. At the base of the swollen area the sac is speckled with spots of yellow-brown increasing in depth of color and size as the tube is reached. Some specimens have been found in which this speckling continues even on to the nutritive portion embedded in the snail's liver. The color of the developing pigment in the sacs begins as a light brick-red, changing to a red-brown, and in old sacs to a deep rich mahogany-brown. In the red-brown specimens the distal end of the sac becomes rather pointed in old individuals and even when fixed has a conical shape. The tube reaching back to the liver is very long and elastic. It will stretch about 4 times the length of the sac and when parted from the liver is about twice the length of the sac. The portion in the liver is profusely branched and the branches are filamentous.

In all the snails kept in the laboratory for daily observation, not a single brood-sac was shed. Snails kept in an experimental plot in which conditions of water, shade, soil, and vegetation were made as near normal as possible shed many brood-sacs. The first sac found in the plot was discovered about 8:30 p.m. The area had been watered at 5:30 p.m. This shed sac is reproduced as figure 4. It will be noticed that the snail must have come out of hiding below the dried leaf stalk. In doing so it presumably ruptured the tentacle. The sac slowly emerged and the snail crawled up dragging the sac until it caught on a leaf stalk, causing the tube of the brood-sac to break free from the liver and adhere to the stalk. This has been the usual position of the shed sacs, most of which have been secured in the early morning from the experimental plot.

It is yet to be determined experimentally whether birds do actually assist in the emergence of the brood-sac or whether the emergence is caused by vegetation tearing the extremely thin, stretched tentacle. The latter method appears more plausible. Wesenberg-Lund was unable to observe a bird in the act of snipping a tentacle. To test the former theory I snipped a tentacle which contained a very active red-brown sac. The sac retracted. When it did resume active pulsations it did not find the hole made. In one hour the snail was dead.



EXPLANATION OF PLATE

1. Brown sporocyst, complete.
2. Orange sporocyst, (brood-sac only).
3. Green sporocyst. Attached to liver of snail. The smaller sac has only red-brown pigment.
4. A naturally shed red-brown sporocyst sac.
5. Red-brown sporocyst, (brood-sac only).

That the brood-sac can exist for some time out of the snail is shown by the following. The largest snail we have found this season and containing the largest sac yet found by us was photographed. The snail was then removed from the shell and the brood-sac allowed to emerge, backward through the puncture made. The sac was placed in normal saline and photographed at 11 a.m. (Fig. 5). It was felt that by waiting for some time, the sac would tire from its active movements and become quiet. At 5:30 p.m. when the photo-flood lights were turned on for a second exposure, activity was too great to allow for even a short time exposure. It was then killed with alcohol and the picture taken. Dissection revealed 194 mature, encysted metacercariae. Thus it is thought possible for the brood-sac to emerge under wet conditions and even drop into water and simulate the appearance of an insect larva for a considerable time. In the field, when picking up parasitized snails, many sacs have dropped from the tentacles because of pressure of the fingers. It may be that, in wet weather or when a snail is immersed in water for some time, the absorption of water causes pressure sufficient to expel the brood-sac.

The first sac usually reaches maturity in the left tentacle. The second then goes into the right and if a third is ready it takes a position over the radula. A snail which contained a large sac in its right tentacle was abnormal to the extent that its neck was enlarged with a yellow mass. Thinking that this might indicate a new species the snail was dissected. It had a large sac in the right tentacle, a second mature sac near the liver while the yellow mass was a very large sac that had doubled back upon itself at the base of the left tentacle with its colored distal end extending back as far as the liver. The length of the sac was over 5 cm. No metacercariae were present in the portion turned back. Before fixing, an attempt was made to shake the metacercariae down into the tip but without success. Another snail with three pulsating sacs, had one in the right tentacle and two in the left.

The Green Sporocyst: The brood-sacs of the green sporocysts are much more slender and more numerous per snail than the red-brown. Also noticeable is the much shortened tube to the liver. This tube has less tensile strength than the brown, and is easily broken from the basal portion in the liver. The colors deepen with the age of the brood-sac.

On the distal end of the slender sac are wart-like protuberances colored bright, deep red to red-brown. At the very tip the protuberance is much enlarged. An area of mottled green on a colorless background extends for a short distance backward from the tip. This area also has small round, red spots some of which are associated with opaque oval white spots. Then follows a broad band of green, below which are longitudinal bands of the same green alternating with white bands

or spots. According to the age of the brood-sac, this green changes from a chlorophyll green, to a blue green, to a jade green and in much advanced sacs, a deep olive green. Continuous with the green stripes and about the same length are stripes ranging from red-brown to a very deep red-brown. Between these green and brown stripes the tissue appears whitish but not opaque. The remaining two-thirds of the sac is a canary yellow with prominent light chrome yellow rings. When the anterior of the sac shows olive green, these basal rings also show flecks of dark olive green pigment. One snail showed 5 sporocysts as described above and a sixth that had produced no sign of green but had its red-brown pigment partly formed (Fig. 3).

The Orange Sporocyst: The distal end is crowned with very numerous, small, red-brown, wart-like protuberances. Below this crown is an area of deep yellow closely streaked with orange, the area appearing orange to the unaided eye. Then follows a broad circular band of red-brown, at about $\frac{1}{3}$ of the length from the distal end. The rest of the sporocyst is colorless except for about ten narrow longitudinal stripes of light brownish red, beginning at the base of the swollen sac and fading out about $\frac{1}{3}$ of the distance anteriorly. A noticeable constriction clearly seen in the photograph (Fig. 2) was always present near the proximal end of the sac. The diameter and length of the sac closely resembles the red-brown sacs.

The Brown Sporocyst: A snail observed in the field, on the afternoon of July 13, had a sporocyst marked only with one dark ring. Dissection that evening revealed what is presumed to be a fourth species of sporocyst. In length it was shorter than the red-browns but about the same in diameter. The observed ring was less than a fourth of the distance from the distal end, appearing to the eye as a distinct ring of dark mottled brown pigment. The much flattened crown was distinctly white. Between the crown and the ring was a coarse scalloping of light brown making a star-shaped pattern with the white crown as the center. A few distinct, brown, elongated spots adjacent distally to the rings were observed to pulsate before the pulsation occurred in the remainder of the sac. The lower portion of the brood-sac was speckled with very small light brown spots. Fearing that this particular sac might be only an abnormal red-brown having had its terminal portion injured and thereby robbed of its distinct red-brown bands, the basal, liver part of the sporocyst was carefully dissected free from the liver. This operation showed that the tube from sac to liver was decidedly shorter and thicker than in the red-browns, and that the nutritive portion on the liver was of a new character (Fig. 1). The red-browns have decidedly filamentous branches. In this brown form the branches were stubby, pouchy, of a form I have never observed in the hundreds dissected.

Furthermore the young brood-sacs, already apparent, were themselves flat-crowned even as the mature sac. Measurements of the metacercariae also show a difference. I feel sure that further finds will bear out the contention that we have here a fourth species. The orange sporocyst has been described on one specimen, but it is so different that there appears no doubt of its being a new species.

A list of our collection of multiple infections with mature sporocyst sacs recently preserved follows:

Snails	Red-brown	Green	Orange	Brown
4	1	1		
1	3			
8	2			
3	3			
1	4			
1	2	2		
1		2		
2		3		
2		5		
1		6		
1			1	
1				1

The number of mature metacercariae per mature sac:

5 green sacs averaged 120.4, maximum 164

15 red-brown sacs averaged 97, maximum 194

*Measurements, in microns, of the encysted metacercariae
after preservation*

	Number of metacercariae in sac	Av. Length	Av. Width	Diam. Ant. Sucker
Red-brown	119	966	535	302
	64	948	598	339
	39	977	515	297
Green	152	506	286	209
	162	453	286	207
	122	444	315	211
Orange	?	1005	557	374
Brown	?	792	506	176

DISCUSSION

Magath (1920) described *Leucochloridium problematicum* from Fairport, Iowa. Up to the present this is the only description of the

sporocyst found in the United States. Magath gives a colored plate of the sporocyst and says, "The distal half of the sporocyst is banded with deep golden red bands which show very accurately in figure A (Plate). Some of these are darker than others and the lighter ones tend to be more yellow. No bands appear beyond the distal half. The proximal $\frac{1}{4}$ is flecked with bronze spots, minute in size, but readily seen. These flecks are also present for a short distance on the tube projection."

It is possible that the species described by Magath is the same as the red-brown described in this paper, although he must have been describing an immature sac. His colored plate showing the three distinct rings does not agree exactly with my specimens. His statement of the length of active cercariae as 2.2 mm. differs from our measurement of encysted larvae which average .950 mm. In the largest sac containing 194 metacercariae the length of larvae reached 1.808 mm. The description by Magath of a constriction in the tube below the sac, is of no significance. Many of the complete sporocysts in my collection show cercariae crawling up the tube to encyst in the brood-sac.

No attempt is made to name these new forms of sporocysts. Since they are larval stages and the adults will no doubt be found in birds, it is advisable to determine the appropriate bird host through feeding experiments, and then follow McIntosh in using the generic name of the bird as the species name for the *Leucochloridium* if they cannot be assigned to known species.

E. L. Cheatum (unpublished field notes) has found *Leucochloridium* in three species of birds from the Erie Marsh and is making a survey of other birds from the vicinity. It will require feeding experiments to determine the exact life histories. More extensive field work over a larger area will undoubtedly reveal many new color combinations in the mature sacs. All the adults described by McIntosh from the Douglas Lake area should be restudied for sporocysts stages. Many of his adult forms may however be from sporocysts without visible sacs in the tentacles of the snail host.

The paper by Wesenberg-Lund on the Biology of *Leucochloridium* has been a source of great stimulation.

My grateful appreciation is extended to the students and associates who have helped in the gathering of snails and have discussed the many questions and problems that have arisen in the course of this study.

SUMMARY

Field and laboratory observational data on *Leucochloridium* are presented.

Four new sporocysts are described, having the following colors, red-brown; green, red-brown, and yellow; orange; brown.

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THREE NEW SPECIES OF TICKS, *ORNITHODORUS*
(ACARINA, IXODOIDEA)

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Some time ago, Major L. H. Dunn presented me with three species of ticks that he thought were undescribed. A search through the literature confirms his belief and the following descriptions are offered.

Ornithodorus dunni n. sp.

ADULT. (Plate I, figs. 1 and 2). Body flat, oval, slightly pointed at anterior end, broadest back of fourth pair of legs. Length 3.4 mm.; extreme width, 2 mm. Sides of body gently curving; posterior border broadly rounded; margin of body more or less rounded, angular points of "cells" giving it a somewhat saw-like edge. Dorsal surface mammillated, with numerous rounded to oval discs arranged as shown in photograph. *Venter*. Ventral surface with many circular discs more or less arranged in radial lines; these discs most numerous directly behind post-anal furrow. Anus located posterior to hind coxae, surrounded by a smooth anal ring, $185\mu \times 160\mu$; anal flap with four spines each. Supra-coxal folds distinct and prominent; eyes absent; coxal folds well marked; pre-anal furrow distinct; post-anal furrow strongly developed and extends almost to margins of body. Spiracles situated above supra-coxal folds and directly above fourth pair of coxae; peritreme crescentric. Genital opening between posterior ends of coxae I. Capitulum relatively stout, free, reaching almost anterior margin of body; situated in a well marked camerostome with movable cheeks; basis capituli ventrally with two pairs of long stout setae (Fig. 1); palps free, gradually tapering; articles 1 and 3 about equal, 2 slightly shorter; hypostome short barely reaching apex of article 2 of palp; rounded apically; dentition 2/2, the large teeth confined to a small area behind apex; posterior to large teeth a few small denticles and apex bears minute teeth (Fig. 1); chelicerae narrowly elongate, much longer than hypostome; digit with two short recurved teeth (Fig. 2).

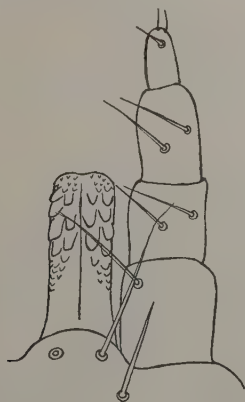


Fig. 1. Hypostome and palp of *Ornithodorus dunni*. $\times 100$.

Legs all about same size, slender; coxae, except I, contiguous and about equal in size; all arise under coxal fold; tarsi without distinct humps; tarsus IV with many spines and a single stout spine about middle of dorsal surface (Fig. 3); claws stout; pulvilli absent.

NYMPHS. From a study of cast skins and various stages before me there are at least two nymphal stages. Stages measure, when apparently replete, 1.5 and 2 mm. respectively. Genital apertures closed in both nymphal stages. No marked characteristics to separate two nymphal stages or nymphal stages from adult except size and closed genital opening. In first nymphal stage capitulum partially withdrawn behind anterior margin of body and beginnings of camerostome evident; hypostome, as compared with that of larva, remarkably shortened, bluntly rounded apically; dentition 2/2; larger recurved teeth now restricted to about distal half of hypostome. In second nymphal stage no marked changes.

LARVA. Newly hatched larva 0.64 mm. from tip of the hypostome to posterior end of body; remarkably elongated hypostome (Fig. 4, a) 0.2 mm. in length; width of body 0.28 mm. Hypostome narrowly elongate, sharply pointed at apex with a dentition of 2/2; lateral teeth very long and apical portion lacks denticles; base of hypostome with two short spines; chelicerae slightly longer than hypostome; digit quite similar to that of adult though more elongate; palps free and with few spines. Fully gorged larva extremely distended, 1.28 mm. in length.

THE EGG. In this collection are numerous eggs laid by the confined adults. The egg is spherical, 0.3 mm. in diameter. The larva, when ready to hatch, lies in the egg-shell like a ball, the capitulum curved under the body. On emerging it assumes a most elongate, almost linear shape and the long legs seem well adapted for rapid movement.

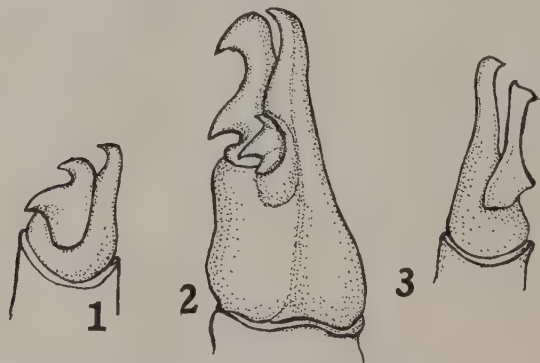


Fig. 2. Digits of the chelicerae. 1. *Ornithodoros dunni*. 2. *O. brodyi*. 3. *O. aztecii*. All magnified 300 times.

HOSTS. This tick was collected in the larval stage on the Little Bull Bat, *Dirias albiventer minor* (Osgood), which was captured in a mango tree in the yard of the Panama Hospital, Panama City, on March 17, 1931. The bat was placed in a bag and during the next few days 32 larvae dropped, fully engorged. The larvae molted, and were carried through the nymphal stages to the adult. Larvae of this tick were also taken on the same species of bat at Summit Canal Zone, on Sept. 30, 1932. This tick appears to be restricted to this one species of bat.

Described from a number of larvae, nymphs and nymphal skins and a series of adults. Types in collection of Cornell University.

This species falls into that group which possess movable cheeks or lateral wings to the camerostome (i.e., with *O. talaje* and its varieties, *O. venezuelense* Brumpt, *O. asperus* Warburton and *O. amblus* Chamberlain). It can be easily recognized by its small size (the smallest so far recognized as belonging to this group), the numerous, somewhat radially arranged discs on the dorsum and venter, the numerous small mammillae and the entire absence of humps on the tarsi I and IV. Its restriction, as far as known, to but one species of bat may also be of aid in identification.



Fig. 3. Tarsus I and IV of *O. dunni*. $\times 50$.

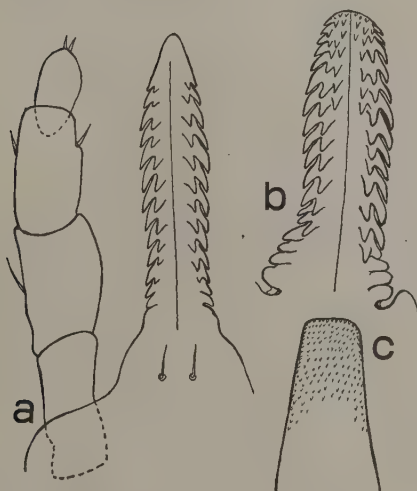


Fig. 4. a. Palp and hypostome of larva of *O. dunni*. b. Hypostome of larva of *O. azteci*. c. Tip of hypostome of nymph of *O. brodyi*. All $\times 200$.

Ornithodoros azteci n. sp.

ADULT. Adult (fully gorged as far as can be determined) 4.5 mm. in length by 2.4 mm. in maximum width; in outline pyriform (Plate I, Figs. 3 and 4), broadly rounded posteriorly and gradually narrowing from behind fourth pair of legs to a rather sharply pointed apex. Dorsal surface somewhat rounded and lacks evident discs. Integument, both on dorsal and ventral surfaces, distinctly mammillated with very minute spines between the mammil-

lae; spines more evident on ventral surface, especially between legs. *Venter*. Pre-anal groove not very evident; coxal and supra-coxal folds prominent; median and post-anal grooves well developed; eyes absent; spiracular openings ovoid, $160\mu \times 80\mu$; peritreme semilunar in shape; anal frame longer than broad ($260\mu \times 250\mu$), each valve with eight short spines; coxae II, III and IV contiguous and about equal in size; coxa I distinctly separated from II and somewhat larger; genital opening crescent-shaped with prominent lips, located between coxae I. Capitulum located in a deep camerostome with prominent lateral, flap-like borders which move as upon a hinge and protect capitulum; these lateral flaps provided with short, peg-like spines on their free margins. Basis capituli about as broad as long, heavily chitinized; hypostome as described for nymph except that denticles are smaller, not arranged in such definite rows and appear fewer in number; two setae at base of hypostome; palps as long as hypostome; joint 1 twice as long as 2; longer than 3; 4 short and pointed and with several short, apical spines; all joints of the palps with long setae.

Legs not varying much in size; IV is the longest; II and III of about equal size and length; I is the shortest; tarsus I and IV (Fig. 5) each with a single hump; claws stout; pulvilli absent; all legs with numerous short setae and scattering long spines.

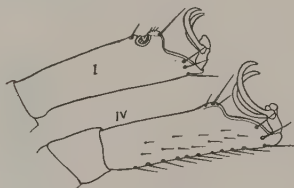


Fig. 5. Tarsus I and IV of *O. asteci*. $\times 50$.

NYMPH. From material before me there appears to be only one nymphal stage. Body elongate, sides sub-parallel, and strongly constricted in front. Length (unfed) 2.88 mm.; extreme width 1.6 mm. Coxal and supra-coxal folds well developed; median and transverse post-anal grooves distinct; pre-anal groove present but not so well marked as in adult; integument has lost all indications of parallel transverse striations (so prominent in larvae) except at anterior end and now appears distinctly mammillated with minute spines between mammillae. Capitulum located in a deep camerostome as in adult; hypostome appears as a very thin, slightly chitinized membrane with only minute denticles arranged in transverse rows on apical portion (Fig. 4, c); chelicerae elongate, sharply pointed shafts terminating in pointed digits (Fig. 2, 3); palps as long as hypostome; joint 1 about twice as long as 3; 2 shorter than 1; 4 short, pointed, with several apical spines.

LARVA. Recently hatched larva narrowly elongate; length 0.88 mm. as measured from apex of capitulum to posterior margin of body; capitulum measures 0.44 mm. Body bears rather numerous stout hairs with minutely spinous margins; integument shows, under high magnification ($\times 400$), very fine parallel lines running transversely, forming a more or less definite pattern; striate condition appears much more distinctly in mature larva and resembles that of *O. megnini*. Hypostome heavily toothed (Fig. 4, b); dentition 2/2; apex with several rows of minute denticles; chelicerae as long as hypostome terminating in a two-toothed article; palps as long as the hypostome; joints 1, 2 and 3 of about equal length; joint 4 is very short and bears numerous short, pointed spines; scattered spines present on joints 2 and 3. Fully gorged larva measures 1.68 mm. in length.

THE EGG. Egg is practically spherical and measures 0.56 mm. in diameter.

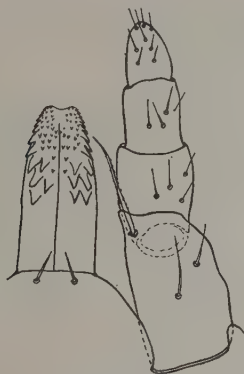


Fig. 6. Hypostome and palp of *O. brodyi*. $\times 66$.

HOSTS. Bats, *Hemiderma perspicillatum aztecum* (Saussure); Summit, Canal Zone, Nov. 12, 1930; this bat was found to be heavily infested with larvae and the nymphs and adults were present in the cracks and crevices of the culvert where the bats roosted in masses. Also taken on this same bat in a cave at Taboga Island, Panama Bay and on the vampire bat, *Desmodus rotundus murinus* Wagner. This tick has also been taken in the cracks and crevices of Chilibrillo Caves and in other caves and culverts in Panama and the Canal Zone.

TYPES. Described from larvae, nymphs and adults and cast skins. All in the collection of Cornell University.

Like the preceding species *O. azteci* falls in the group with movable checks or lateral wings to the camerostome. It is readily distinguished by its characteristic shape, size, lack of visible discs on the integument, the structure of the hypostome and the humped tarsi. Its nearest relative appears to be *O. venezuelense* Brumpt, from which it can be separated by its shape, the distinctive integument without discs and with much smaller, more closely packed mammillae, the much more prominent and deeply incised post-anal groove and the remarkable reduction of the hypostome in the nymph and adult.

Ornithorodius brodyi n. sp.

ADULT. Length (fully gorged female) 6 mm.; maximum width 4 mm.; sides of body parallel, broadly rounded behind and sharply conical in front (Plate I, Figs. 5 and 6); frequently slight lateral constrictions in region of third pair of legs; general color dirty yellow or almost black when replete (alcoholic specimens); legs dirty yellow to straw yellow in color. Dorsal and ventral surfaces covered with closely packed, small mammillae with short hairs or spines between them; discs absent. Supra-coxal and coxal folds well marked; pre-anal groove present; post-anal median and transverse grooves very prominent even in replete individuals; eyes absent; anal frame longer than wide ($320\mu \times 240\mu$); genital opening immediately behind coxae I; spiracular opening appears as a narrow crescentric slit; peritreme a large crescent-shaped sclerite. Capitulum relatively small, well protected in a deep camerostome with prominent movable cheeks and apical lobe; palps 4-jointed, free,

tapering; joint 1 longest, 2 and 3 about equal, 4 shorter than any of others (Fig. 6); hypostome shorter than chelicerae; dentition 2/2, the apical part with numerous small teeth (Fig. 6); chelicerae stout, terminating in an article with two digits (Fig. 2, 2).

Legs long and slender; coxae II, III and IV contiguous; coxa I distinctly separated from II; coxae gradually diminish in size from I to IV; tarsus I with a poorly developed hump; tarsus IV without any well developed hump (Fig. 7). All legs provided with numerous spine-like setae; claws large; pulvilli practically absent.

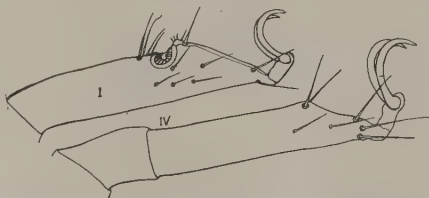


Fig. 7. Tarsus I and IV of *O. brodyi*. $\times 50$.

NYMPH. From the material before me there appears to be two nymphal stages. Fully gorged nymph (last nymphal stage) 4.5 mm. in length by 2 mm. in width. In general shape nymph closely resembles adult with no marked difference except size and absence of genital opening. Capitulum practically identical with that of adult except that at base of hypostome there are two very long spines that occupy the same position as the two short ones do in the adult.

LARVA. Fully gorged larva practically spherical in shape, the long legs and capitulum projecting far beyond margins of body; length, exclusive of capitulum 2.3 mm.; capitulum 0.64 mm.; extreme width 2.3 mm. Hypostome of larva practically identical with that of *O. asteci* (Fig. 4, b). Integument of partially gorged larva with beautiful arrangement of fine parallel lines running in various directions on body.

THE EGG. Eggs obtained in association with this tick practically spherical in shape and measure 0.48 mm. in diameter.

HOST. Larvae of this species were taken on the Short-tailed Bat, *Hemiderma perspicillatum aztecum* (Saussure) on March 30, 1931, in the Chilibrillo Caves, Panama, by Mr. A. L. Brody. Later he found nymphs and adults in crevices of the walls and ceilings of these caves. Eggs were also found in association with the adults.

TYPES. Described from 6 adults, 3 nymphs and 3 larvae. All in the collection of Cornell University.

Like the two preceding species *O. brodyi* falls in the group with movable cheeks to the camerostome. The shape, size, the marked apical lobe, the very elongate, movable cheeks, the integument without discs and crowded with smooth, rounded mammillae, and tarsi I and IV without evident humps mark the species as distinctive. The absence of humps on tarsi I and IV readily separates the species from *O. asperus* Warburton and the finely mammillated integument without discs separates it from *O. talaje* (Guérin-Meneville) and its varieties and from *O. venezuelense* Brumpt.



EXPLANATION OF PLATE

Plate I. Figs. 1 and 2. *Ornithodoros dunni*. Dorsal and ventral views.
Figs. 3 and 4. *Ornithodoros axteci*. Dorsal and ventral views.
Figs. 5 and 6. *Ornithodoros brodyi*. Dorsal and ventral views. All
figures magnified 6 diameters.

ALLANTOIN, A CONSTITUENT OF MAGGOT EXCRETIONS, STIMULATES HEALING OF CHRONIC DISCHARGING WOUNDS*

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One of the distinguishing features of the maggot treatment of infected wounds is the rapidity with which the wound usually heals. This has been stressed both in the literature and in statements (7) received from surgeons who have used this treatment. It has already been shown (1, 2, 4, 5, 10) that, in feeding upon necrotic tissue and destroying bacteria, maggots put the wound in a condition more favorable for healing. The rapid rate of healing indicated, however, that maggots are more than merely scavengers and that they excrete something into the wound which directly stimulates the process of healing.

An investigation (8) of maggot excretions was begun to determine, if possible, whether any substance with healing properties could be found. Any direct healing effect by maggots would, no doubt, be the result of an involuntary act in connection with their feeding or metabolism. An investigation of the urinary excretions, which contain the products of metabolism, was therefore attempted first. The search was greatly simplified by an analogy as described next.

Scattered throughout biological and medical literature are reports that healing of wounds has been stimulated by the application of macerated embryonic tissue or extract. A conspicuous thing about those experiments was that the embryos used typically possessed a well-developed allantois containing allantoin; and in the present investigation it was believed that this might be significant, as most vertebrates and invertebrates, possibly including insects, excrete allantoin as a waste product of metabolism. No mention could be found, however, that the healing effects of embryonic tissues or fluids were due to this substance and, in fact, nothing as to the healing value of allantoin was found at that time in any works on pharmacology, therapeutics, or pathology. Interest was added to the investigation by the finding of an article written by Macalister (3), twenty-three years ago, stating that he had successfully used allantoin obtained from the roots of comfrey in the treatment of chronic ulcers.

By the following procedure maggot excretions were readily obtained in sufficient quantity for the separation, purification, and identi-

* Contribution from the Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

fication of allantoin. Several thousand nearly full-grown maggots were reared under both aseptic and non-sterile conditions. These were placed in glass funnels stoppered with cotton, and half filled with small glass macerating balls to prevent too great massing together of the maggots. The maggots were occasionally sprayed lightly with water from an atomizer to facilitate excretion and drainage. Various methods of preventing the escape of maggots from the funnels are, of course, possible. The excretions were allowed to drip into beakers for three to four hours, until a sufficient quantity was obtained. With the sterile maggots, aseptic technic had to be used through the collecting process.

From 20 c.c. of both the sterile and non-sterile liquid, allantoin was separated into the crystalline condition, purified, and finally identified by comparing its melting-point, its mixed melting-point, and its optical crystallographic properties with those of an authentic sample.*

The effect of allantoin in stimulating healing in wounds was then carefully tested in cooperation with hospitals in Washington, Pittsburgh and New York. Fortunately this substance can be obtained commercially and a supply was purchased. It could not, of course, be applied to the wound as maggots do it. When maggots are placed in an infected wound they feed continuously upon the necrotic tissue present, and while feeding and growing they constantly excrete allantoin in minute quantities within the wound. They thus constitute a reservoir with a small, steady supply. In the allantoin treatment, gauze dressings saturated with the solution were placed in the wound. Methods of preparation and application are briefly described later.

The cases in which this treatment was given were those with chronic non-healing wounds with unhealthy, inactive tissue, poor in circulation and discharging pus. Such wounds are of common occurrence and are resistant to healing. They included cases of chronic ulcers, of failure to heal after extensive burns, and also of wounds in which only a slight discharge was present but without indications of healing. As a wound heals, new pinkish tissue called granulation tissue, with ample blood supply, develops and fills in the wound and is finally covered with epithelium. In non-healing wounds there is not only the presence of infection with discharge of pus, but the tissues lining the wound are sluggish, pallid in appearance, and non-resistant to the invading bacteria. Healthy, active granulation tissue acts somewhat as a barrier to pus-forming organisms.

* The isolation and identification of allantoin in these experiments were performed by Dr. E. P. Clark of the Division of Insecticide Investigations of this Bureau and Dr. G. L. Keenan of the Food and Drug Administration, U. S. Department of Agriculture.

After the first few treatments with allantoin small areas of shining, pinkish granulation tissue could be seen growing in the wounds. This was followed later by a general development of this tissue. It had an abundant blood supply and the appearance of healthy tissue. In soft-tissue wounds, as described, the allantoin treatment was found to be effective in cleansing the wound and producing healthy granulations. In cases of infected bone and bone marrow the rate of healing was not, however, found to be so rapid as when maggots were used initially. There is no doubt that living maggots play a definite rôle and are especially valuable in such deep-seated infections. It will be remembered that maggots are very effective in removing dead and dying tissue from the wound and in reducing the number of pus-forming bacteria (2, 5, 10). It is not claimed that the allantoin treatment can be substituted for all the activities of maggots in the wound. The object of this investigation was, as previously mentioned, to determine if maggots excrete any substance into the wound which promotes healing.

Allantoin is harmless and, besides stimulating new growth, has a soothing effect. It has no taste or odor and does not stain the skin or clothes. It is also quite inexpensive, and a daily application of about 100 cc. of solution to a wound of moderate size costs approximately ten cents. In preparing it for use a satisfactory method is to heat a flask of sterile water to near the boiling-point, then add the allantoin and gently heat without boiling until the crystals are dissolved. This should be done with reasonable aseptic precautions, as the solution cannot be autoclaved or boiled without a chemical change occurring. The strength of solution used in the present tests was at the rate of 2.5 grams of allantoin to 500 cc. of water. It was made up in quantities for about one week's use. It should not be chilled in the refrigerator as the allantoin would tend to crystallize. It is better kept out in the laboratory but away from excessive sunlight.

The application of allantoin appears to stimulate a local, rather than a generalized, granulation. This is fortunate, as the extent of new growth can be controlled. In a deep wound where it is necessary to promote healing from the bottom upward and to restrict lateral growth, this can be done by applying the solution in a small packing to the base of the wound and covering the sides very lightly with vaseline. Where general granulation is required, the wound is loosely filled with gauze dressings well soaked with solution. Some of it is also poured into the wound. A wet pack is then laid on top to retard drying and covered with one or two dry packs which are fastened down with adhesive plaster. The gauze packing is placed lightly in the wound to prevent adherence to the tissue. This treatment is painless, is easily given, and requires but little time. Dressings are re-

moved before drying. No chemicals are used in the wound during the treatment, and before initial use the wound is well washed with sterile water.

The origin of allantoin is of interest. It is naturally-occurring component of the urinary secretions of animals below man and the man-like apes. It is a substance which results from the metabolism of the cell nucleus and is related to uric acid, from which it is derived in nature through the action of an enzyme called uricase. Allantoin is also widely distributed among plants, where it is found chiefly in the embryos of seeds.

As this substance is a metabolic product, its removal from the blood is effected through the action of the kidneys. With insects, however, metabolic waste materials are not eliminated directly but, as the Malpighian tubules connect with the intestine, such materials pass out through the intestinal tract. This brings about a curious combination of characteristics of maggot excretions. On the one hand, in a search for the reason why the number of bacteria in the wound decreases under the maggot treatment, Robinson and Norwood (5, 6) found that maggots destroy bacteria which are taken into the stomach with the food. The intestine in every instance was sterile. An undetermined substance in the alimentary canal thus has the power to destroy certain species of bacteria from infected wounds. On the other hand, the present investigation (8) shows the occurrence of allantoin which has the property of stimulating growth of tissue, a characteristic of an opposite nature. These two substances pass along the intestine together. Simmons (9), in an investigation of the bactericidal properties of maggot excretions, has found the excretions to have a powerful bactericidal action upon cultures of various species of pus-forming bacteria.

Materials which arise from the metabolic processes have until recently been regarded largely as waste substances and presumed to be excreted as such from the body. There is a growing tendency, however, to believe that some of these products are reutilized by the cells. Confirmation of this is found in the present experiments.

An increase in the number of leucocytes in the blood has frequently been found associated with the presence of blood parasites, and the increase might be attributed in part to the secretion of purines by these organisms in the blood. The formation of galls in plants is also possibly due to the action of allantoin or some related purine excreted by the parasite.

SUMMARY

An investigation of the unusually rapid healing of wounds under the maggot treatment has been made, and a substance has been isolated

from maggot excretions which has the property of stimulating healing in infected wounds. It has been identified as allantoin, a product of metabolism of the cell nucleus of both plants and animals. The excretion of this substance into the wound is apparently one of the factors contributing to the remarkable healing effects obtained in maggot therapy; but the claim is not made that allantoin can be entirely substituted for the use of maggots. Allantoin is of common occurrence and can be obtained commercially. It is harmless and soothing and has no odor or taste. Treatment of wounds with this material is simple, painless, and inexpensive. A method of preparing allantoin solution and of using it is described.

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A NEW TREMATODE, *PROBOLITREMA CALIFORNIENSE*,
FROM THE COELOM OF THE STING RAY,
*MYLIOBATUS CALIFORNICUS*¹

HORACE W. STUNKARD

Six trematodes were found by G. E. MacGinitie, of the California Institute of Technology, in the coelom of four sting rays dissected on July 20, 1927. The specimens, mounted *in toto*, were referred to me in June, 1934, for study and identification. According to data supplied by MacGinitie, the rays were taken from Elkhorn Slough, a salt water estuary of Monterey Bay. The worms were not attached, nor were there any spots on the viscera or body wall to indicate that they had been attached. The specimens belong to the genus *Probolitrema* Looss and appear to represent a new species. Grateful acknowledgment is made to Mr. MacGinitie for the privilege of examining and describing this material.

HISTORICAL

Lopez (1888) described a trematode from the body cavity of *Acanthias vulgaris* as *Distoma richiardii*. A more detailed description of this species was given by Monticelli (1893), who reported it from *A. vulgaris*, *Mustelus vulgaris*, and *Myliobatus aquila* in the Mediterranean. Ofenheim (1900) described similar trematodes, collected by Schauinsland from the coelom of *Aëtobatus narinari* during an expedition to the Pacific Ocean. For these worms he proposed the name *Anaporrhutum albidum*. In the same paper Ofenheim described other specimens, collected from the coelom of a species of *Scyllium* taken in the Atlantic Ocean near Kapstadt, and identified as *D. richiardii* of Lopez. Because of morphological similarity between these worms and *A. albidum*, he transferred the species of Lopez to the genus *Anaporrhutum* as *A. richiardii*.

Looss (1902) recognized specific differences between the specimens described by Lopez and Monticelli and those identified by Ofenheim as *A. richiardii*. Furthermore, according to Looss, neither of the species is congeneric with *A. albidum*. Accordingly, *Distoma richiardii* of Lopez was removed from the genus *Anaporrhutum* and designated as type of a new genus *Probolitrema*. The specimens studied by Ofenheim and described by him as *A. richiardii* were renamed *Probolitrema capense*. Looss divided the family Gorgoderidae into two subfamilies, viz., Gorgoderinae and Anaporrhutinae. A review and revision of the subfamily Anaporrhutinae was made by Nagaty (1930).

¹ Contribution from the Biological Laboratory, New York University.

More recently Johnston (1934) has described three new species of *Probolitrema* from elasmobranchs taken in Australian waters. *Probolitrema rotundatum* was found in the coelom of the fiddler ray, *Trygonorrhina fasciata*; *P. clelandi* and *P. simile* were collected from the coelom of the gummy shark, *Mustelus antracticus*. Johnston did not state the number of specimens studied, but he reported that all of the material of *P. rotundatum* came from a single host and the other two species were described from a few specimens. *Probolitrema clelandi* and *P. simile* were distinguished by differences in size of eggs and distribution of testes. Concerning these two species Johnston stated, "Perhaps an examination of a large series may show that there are intermediate forms, and that both should be united under the former name."

The specific description of a limited amount of material is a difficult and unsatisfactory undertaking. The observer can usually determine whether the specimen before him is contracted or extended and whether or not it is distorted. If it is alive, certain data are afforded concerning changes in form as movement occurs, but a single specimen gives no adequate concept of the limits of variation which naturally occur in the species. Even the examination of several specimens may not provide an accurate and complete specific description. When descriptions must be made from preserved material alone, which frequently happens in the case of museum specimens, the author is very much handicapped. In the description of parasitic species a further peculiar difficulty is encountered. The former belief in rigid host-parasite specificity has been almost abandoned since experimental studies have shown that one and the same parasite may infest several host species. Moreover, it has been demonstrated that the size, form, and appearance of the parasite may be affected to a considerable degree by development in different host species. The diagnostic features and limits of a species can be stated only when an abundance of material is available. From the examination of a few preserved specimens it is often impossible to distinguish between individual variations and true morphological differences. Consequently the literature is encumbered with many specific names which fall into synonymy as knowledge becomes more complete and precise.

Too often the descriptions of known species are so inadequate that a comparison between unidentified specimens and a given description does not lead to positive determination. Although there is much conformity, the specimens do not agree entirely with the description. This problem is met repeatedly by workers who attempt to identify material. In reporting on such specimens, two alternative courses are available. If agreement is general, the specimens may be referred tentatively to the previously described species. Conversely, if the differences are so pronounced as to render it improbable that the specimens belong to a known

species, it is expedient to designate them with a new specific name, since in practice it is easier to drop a specific name in synonymy than to distinguish between two species which have been confused in a single common description.

The specimens from *Myliobatus californicus* do not entirely agree with any previously described species. Only six preserved individuals are available for study and accordingly it is impossible to determine with certainty their correct specific disposition. The worms differ from both *P. richiardi* and *P. capense* in their smaller size, smaller size of all organs, especially the gonads, and smaller number of testes. They are larger than *P. rotundatum*; the suckers, genital organs and eggs are

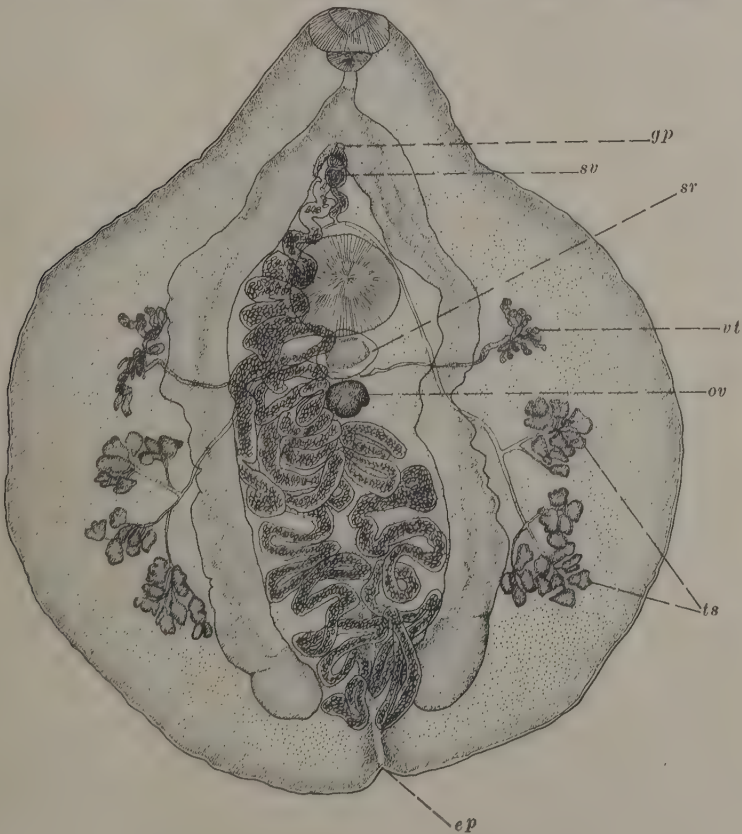


FIG. 1. *Probolitrema californiense*, ventral view.

ep, excretory pore; gp, genital pore; ov, ovary; sr, seminal receptacle; sv, seminal vesicle; ts, testis; vt, vitellaria.

larger, and they differ in form of the vitellaria. They differ from *P. clelandi* and *P. simile* in size of acetabulum, shape of ovary, and in shape, number, and distribution of testes. These features may serve as a differential diagnosis of the present specimens for which the name *Probolitrema californiense* is proposed.

Probolitrema californiense n. sp. (Fig. 1)

Diagnosis: With the characters of the genus *Probolitrema*. Sexually mature specimens, 7-13 mm. long and 5.7-10.2 mm. wide. Acetabulum, 1.2-2.4 mm. in diameter. Oral sucker about 0.75 mm. long and 1 mm. wide. Testes extracecal, 16-26 follicles in each testis. Seminal vesicle divided into two sections. Ovary lobed, 0.35-0.6 mm. long and 0.54-0.925 mm. wide. Vitellaria poorly developed. Eggs, 0.075 by 0.047 mm.

Host: *Myliobatus californicus*.

Locality: Monterey Bay, California.

Type Specimen: In the Department of Lower Invertebrates, American Museum of Natural History, New York.

MORPHOLOGY OF *P. californiense*

All six of the specimens from *M. californicus* are sexually mature. They are circular to oval with the region in front of the genital pore extending forward as an anterior protuberance. The worms are much flattened and so translucent that the internal organs are visible in the whole mounts. They vary in size from 7 by 5.7 mm. to 13 by 10.2 mm. The acetabulum is situated one-fourth to one-third of the body length from the anterior end and measures from 1.2 to 2.4 mm. in diameter. Apparently the size of the sucker varies with the degree of flattening of the specimen. Although the cuticula has been more or less completely lost, there are indications that the anterior, ventral surfaces of the worms bore flat spines.

Digestive System. The oral sucker is terminal or nearly so, with the mouth opening slightly ventrad. The cuticular lining of the sucker may show thickened meridional ridges. The sucker is flattened anteroposteriorly and measures about 1 mm. in width by 0.75 mm. in length. In the smallest worm it measures 0.67 by 0.62 mm. and in the specimen shown in figure 1, it is 1.25 by 0.65 mm. The pharynx is situated immediately behind the oral sucker. Their contiguous faces may be flattened or the pharynx may indent the caudal margin of the oral sucker. The diameter of the pharynx is slightly less than one-half the diameter of the oral sucker. The esophagus is short and its anterior end is crossed by the transverse commissure of the nervous system. The alimentary tract bifurcates just in front of the genital pore and the ceca extend posteriorly almost to the caudal end of the body. The ceca are curved, following the lateral contours of the body, and divide the worm into three longitudinal fields of approximately equal width. In all of the specimens

the ceca are wide and filled with decomposing food material. Their walls are slightly irregular, but not sacculate, and they end blindly.

Excretory System. Only the larger portion of this system could be observed. The pore is terminal, often at the base of a caudal indentation. The vesicle was traced forward on the ventral side of the body about two-thirds of the distance to the ovary but its anterior end could not be distinguished among the uterine coils. Accordingly, it was impossible to determine whether the vesicle is cylindrical or Y-shaped. Nothing could be observed of the collecting ducts or the flame cell pattern.

Male Reproductive System. The testes are extra-cecal, post-ovarian, and multipartite. Each testis consists of 16 to 26 irregularly shaped, lobed follicles disposed in 4 to 6 groups as shown in the figure. There is much variation in the follicular arrangement. Each follicle is drained by a short tertiary duct. These ducts unite to form secondary sperm ducts which unite in turn to form primary ducts. The primary ducts unite to form the vasa deferentia, one on each side of the body, which pass forward and mediad below the intestinal ceca. Just in front of the acetabulum the vasa deferentia unite to form the seminal vesicle. The vesicle is divided into two sections. The posterior section, somewhat longer than the anterior one, is sinuous or coiled and of uniform diameter. The anterior section has a diameter about twice as great as the posterior section and is bent in an S-shaped manner so that from dorsal or ventral view it appears divided into two portions. There is no cirrus sac and a short, muscular, ejaculatory duct leads from the seminal vesicle to the genital pore. There is a single opening to the exterior, situated midway between the oral and acetabular suckers.

Female Reproductive System. The ovary is situated slightly anterior to the middle of the body and it may lie on either side of the median plane. It is usually trilobed, broader than long, and measures from 0.54 by 0.35 to 0.925 by 0.6 mm. The oviduct arises at the median anterior face and passes diagonally forward where it receives short ducts from the seminal receptacle and the vitelline canal. The ootype region is enclosed in the cells of Mehlis's gland. The seminal receptacle is larger than the ovary. It is situated in front and either to the right or left of the ovary, between it and the acetabulum. The vitellaria consist of branched, digitiform follicles situated in the extracecal areas, anterior to the testes. Vitelline ducts pass mediad, ventral to the ceca, and bend dorsally at the level of the ootype where they meet to form the short common vitelline duct which discharges into the ootype. The uterus consists of coiled descending and ascending limbs which almost completely fill the intercecal area behind the ovary. The ascending limb passes forward on the antovarian side of the body to the genital pore.

The eggs are numerous, thin-shelled, operculate. Those which are opaque give an average measurement of 0.075 by 0.047 mm., while translucent eggs are smaller and average 0.065 by 0.04 mm. This difference probably represents the shrinkage caused by fixation.

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ON THE LIFE HISTORY OF *LEPODERMA RAMLIANUM*
LOOSS, 1896, AND ITS DEVELOPMENT FROM A
XIPHIDIOCERCARIA

M. ABDEL AZIM

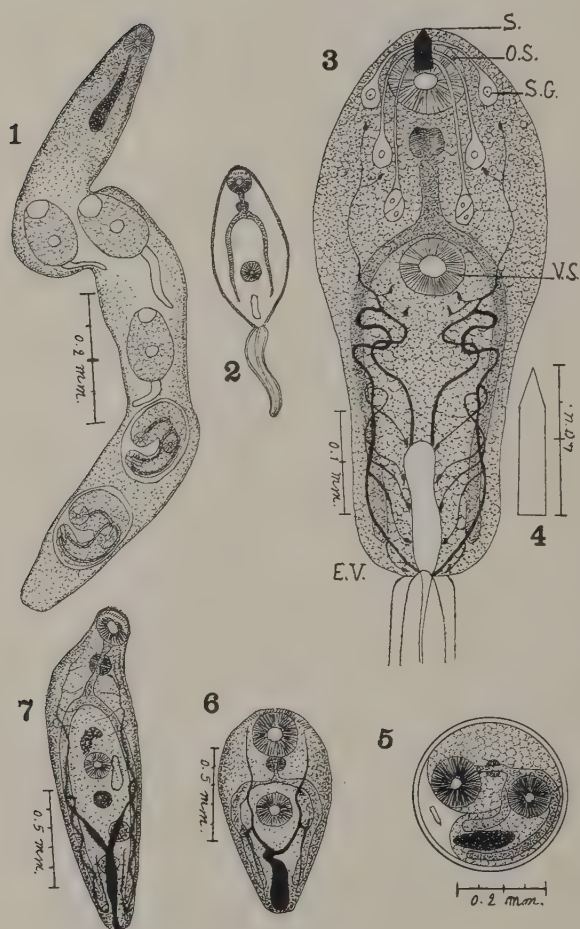
Research Institute and Tropical Diseases Hospital, Cairo.

During the course of investigations on Bilharzia infections among different snails, I came across a peculiar type of xiphidiocercariae liberated from *Bulinus contortus*.

The cercariae were white in colour with elongated bodies and short stumpy tails. They were extremely sluggish and had a marked tendency to sink towards the bottom of the test tube. Specimens a few hours old, drawn from the tube, were surrounded with a rudimentary cyst wall and the tail was partially attached to them. The body was 0.57 mms. in length by 0.22 mms. in breadth. The tail was 0.3 mms. long in extended specimens. The oral sucker was 80μ in diameter and communicated with a well-developed pharynx leading to a thin oesophagus. This bifurcated, anterior to the ventral sucker into two intestinal ceca reaching as far posteriorly as the border of the body. The ventral sucker was 60μ in diameter. The surface of the body was covered with minute spines attaining their optimum size in the region of the oral sucker where they were arranged in concentric rows; then, they became sparsely distributed over the rest of the surface of the body. Its parenchyma was extremely cellular owing to the presence of numerous cystogenous glands, which obscured the minute details of the excretory system. On either side of the body, there were three stylet glands (fig. 3). Each had a separate duct opening in the vicinity of the stylet. This structure (fig. 4) was thin and fragile. It was partially embedded in the parenchyma of the oral sucker, had no marked shoulders and measured 40μ in length; the spine being one-fourth the total length of the stylet.

The excretory system (fig. 3) comprised two tubes; the anterior tube received two groups of tributaries, each with a couple of flame cells which drained the regions of the oral and ventral suckers. The posterior tube received a group of tributaries with three flame cells and another with a single flame cell. Both groups drained the region posterior to the ventral sucker. The two tubes joined each other at the level of the posterior margin of the ventral sucker, forming a thick main tube. The main tube from either side opened directly into the excretory vesicle, which received the main tributary from the tail, at its posterior margin. The formula of the excretory system would be $2 \times (2 + 2) + (3 + 1) = 16$.

The cercariae developed in rediae (fig. 1) about 1 mm. in length

DEVELOPMENT STAGES OF *Lepoderma ramliianum*

- Fig. 1. Redia containing different stages of cercariae.
 Fig. 2. General outline of a cercaria.
 Fig. 3. Body of cercaria showing the details of the excretory system. S. stylet; O. S. oral sucker; S. G. stylet glands; V. S. ventral sucker; E. V. excretory vesicle.
 Fig. 4. Stylet.
 Fig. 5. Cyst with metacercaria and a stylet lying free within the cyst wall.
 Fig. 6. Metacercaria liberated from the cyst.
 Fig. 7. Immature trematode 5 days old.



Fig. 8. Adult *Lepoderma ramlianum* and an egg from an experimental toad (orig.).

0.137 μ in breadth with a well developed oral sucker and a rhabdocele gut. There was no birth pore. Germinal cells, immature, mature free and encysted cercariae were observed in the rediae, also in the tissues of the liver and the hermaphroditic gland of the male. Identical cysts were met with in the thoracic muscles of aquatic hemiptera and the larvae of the dragonfly.

Experiments were carried out to determine if encystation took place in other snails. Three specimens of *Physa acuta* bred in the laboratory and three specimens of *Planorbis boissyi* from another locality were put together with an infected *Bulinus* liberating free cercariae. After two days, dissection of a specimen from each group showed encysted metacercariae in their tissues particularly in the visceral cavity. The cysts were identical to those seen in *Bulinus* snails and showed a stylet lying free within the cyst wall (fig. 5). The diameter of the cyst was 350 μ . With slight pressure applied to the cyst, the metacercariae (fig. 6) were brought out. Each was 1.1 mm. in length by 6 mm. in breadth. The excretory vesicle was markedly Y-shaped, owing to the considerable increase in the width of the two main tubes joining the bladder.

The metacercaria underwent metamorphosis, which was complete in 15 days. Experience showed that cysts below this age failed to develop in the final host.

As these experiments were carried out in the winter months of January and February, the snails had to be kept in aquaria maintained at a constant temperature of 22° C. The time necessary for the development of the metacercariae would thus vary according to the temperature of the atmosphere.

The presence of a Y-shaped excretory vesicle, with the stem being much longer than the two limbs, suggested that the parasite was a member of the family Lepodermatidae Odhner e.p. Baer, 1924. This was proved by further experiments.

On previous occasions, adult and immature trematodes belonging to the genus *Lepoderma* (*Plagiorchis*), were secured from the small intestine of the common toad "*Bufo vulgaris*" caught at "el Marg." As the toads were known to devour snails and insects, they were tried for feeding experiments.

Out of a batch of twenty toads collected from a locality, where *Bulinus* snails were known to be absent, two were separated. A few individuals were killed and proved to be free from trematodes. The feces of the two toads was frequently examined for trematode eggs with negative results. They were given bits of tissues containing cysts from an infected snail. One of the toads was killed after an interval of five days and was found to harbour six trematodes in the same degree of development. Each (fig. 7) measured 1.7 mms. in length. The male genitalia, i.e., the testes and the cirrhus sac were quite visible. Of the female genitalia, only the ovary could be made out. The feces of the other toad showed trematode eggs on the tenth day after feeding. The ova measured $35\ \mu$ by $22\ \mu$. When the toad was subsequently killed, a mature trematode, belonging to the genus *Lepoderma*, was found in the lumen of the small intestine.

Reviewing the literature, revealed that there was no previous record of a xiphidiocercaria from *Bulinus* snails. Nevertheless, a comparison was made between the present species and the xiphidiocercariae on record. It was found to be morphologically different on account of its larger size, the form of the stylet and the presence of innumerable cystogenous glands. The adult parasite (fig. 8) conformed in all the details of its morphology, with *Lepoderma ramlanum* Looss, 1896, described from a chameleon.

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THE LIFE HISTORIES AND CLASSIFICATION OF TWO
ALLOCREADIID-LIKE PLAGIORCHIDS FROM FISH,
MACRODEROIDES TYPICUS (WINFIELD) AND
ALLOGLOSSIDIUM CORTI (LAMONT).¹

DONALD B. McMULLEN

In examining snails collected in the region of the University of Michigan Biological Station, located on Douglas Lake in the northern part of the Southern Peninsula of Michigan, nineteen species of Xiphidiocercariae were found. These cercariae were placed with a wide variety of organisms in order to determine their second intermediate hosts. The metacercariae which developed were then fed to 24 species of animals, representing all classes of vertebrates. Two of the species of these cercariae were in this way proved experimentally to be those of *Macroderoides typicus* and *Alloglossidium corti*. The details of the experimental work and the morphology of the stages of these two species are discussed in the present paper.

The cercariae of both species were found developing in small sporocysts in *Heliosoma trivolvis* and *H. campanulata*. The incidence of infection with the cercaria of *M. typicus* was relatively high and infected snails were collected from several areas in Douglas Lake, Burt Lake, and Mullet Lake. The cercariae were found experimentally to penetrate into several possible intermediate hosts but tadpoles were used in the infection of the definitive host. Experimentally infected tadpoles were fed to *Amia calva* and large numbers of trematodes, identified as *Macroderoides typicus* (Winfield), were recovered from the intestine. The incidence of infection with the cercaria of *A. corti* was relatively high in the snails collected in Munro Lake and lower in those from several areas of Douglas Lake. It was experimentally demonstrated that this cercaria would penetrate into and encyst in dragonfly and mayfly nymphs. The metacercariae from these hosts were fed to small, trematode-free bullheads and adult flukes, identified as *A. corti* (Lamont), were recovered from the small intestine.

THE LIFE CYCLE OF *Macroderoides typicus*, FROM *Amia calva*, AND
THE MORPHOLOGY OF THE DEVELOPMENTAL STAGES

The mature daughter sporocysts of *M. typicus* (fig. 1) contain 1-4 cercariae and measure about 0.36 x 0.13 mm. The cercariae emerged

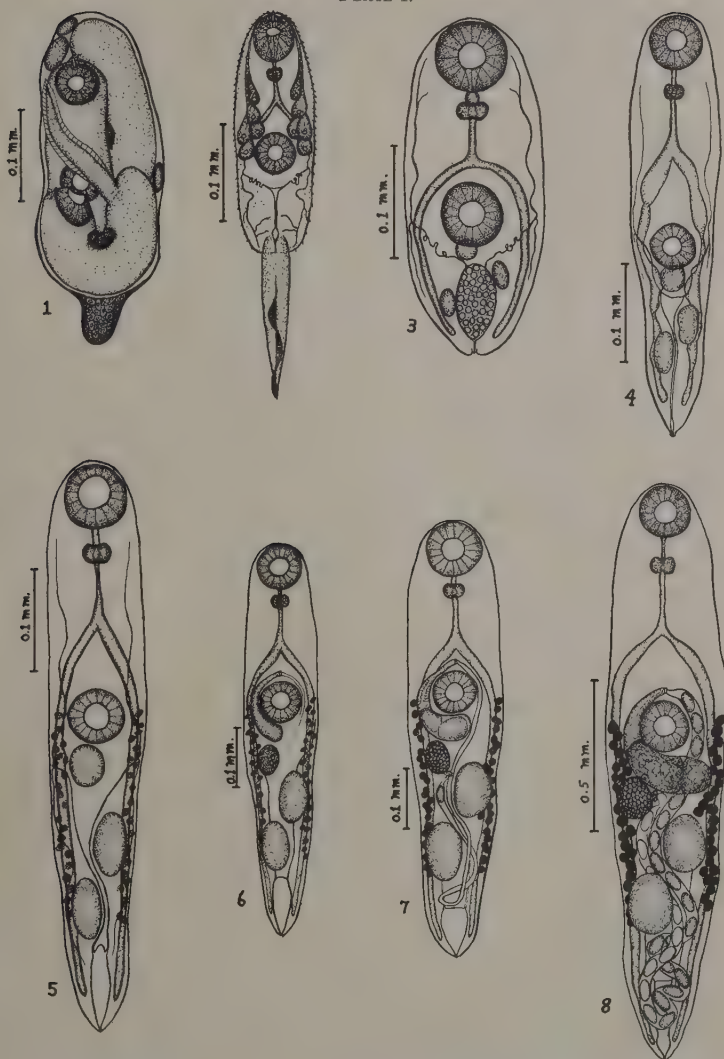
¹ Contribution from the University of Michigan Biological Station and the Department of Helminthology of the Johns Hopkins University, School of Hygiene and Public Health. The writer wishes to express his appreciation to Dr. W. W. Cort, under whose direction this work was done, for many helpful suggestions and criticisms and to Dr. G. R. La Rue for the facilities placed at the writer's disposal at the University of Michigan Biological Station.

in the mid-morning hours and if a second intermediate host was not found they died within a few hours. They were mediocre swimmers and soon after emergence either became entangled or actively penetrated into the mucus secreted by the snail. This lodgement of the cercariae in the mucus was so characteristic of the species that it almost could have been used for identification.

The body of the cercaria (fig. 2) is covered with fine spines that are quite numerous at the anterior end and sparse at the posterior end. The tail is quite extensible, from one-third to longer than the body length, and has a well defined fin-fold that extends from one-half to two-thirds of its length. The stylet is weak and measures about 0.011 mm. in length. At its posterior end a core projects about 0.003 mm. The digestive system consists of a short prepharynx, a muscular pharynx, an esophagus that is longer than the prepharynx, and intestinal ceca that could not be followed beyond the stylet glands. There are five pairs of stylet glands, four grouped in the second quarter of the body and easily seen and the fifth more anterior and difficult to see. The ducts of these glands pass forward along the lateral border of the body and empty close to the base of the stylet. Scattered through the body there is a considerable number of large, refractile concretions. The excretory bladder is I-shaped with a small expansion at the anterior end where the main collecting tubes are attached. These tubes pass anteriorly and each one divides at a level with the posterior border of the acetabulum into an anterior and posterior tubule. The flame cells and the smaller branches of the excretory system were not seen. In an attempt to obtain relatively uniform measurements of the body, tail and suckers, cercariae were killed with formalin, mercuric chloride, Bouin's fixing fluid, and heat. The most uniform results were obtained by placing cercariae under a coverslip with enough water for swimming, and killing them by passing the slide carefully through a flame. At death they were rather well extended and the measurements were more uniform than those from cercariae killed by the other methods. The results obtained in measuring 34 cercariae, killed in this manner, are shown in table 1. It was impossible to measure all the parts in each cercaria, so the number of each of the individual parts measured are given in the table.

This cercaria resembles *Cercaria campanulata* (Brooks, 1930) from *Heliosoma campanulata* collected in the Douglas Lake region. *C. campanulata*, however, has a longer stylet, four pairs of stylet glands and the sporocyst is about six times as large as that of *M. typicus*. The cercaria of *M. typicus* is also similar to *Cercaria hemilophura* (Cort, 1915) from *Physella gyrina* collected near Rockford, Illinois. *C. hemilophura* has a larger stylet, the stylet glands were not seen and the

PLATE I.



Larval and developmental stages of *Macroderoides typicus*, a parasite in the intestine of *Amia calva*.

- Fig. 1. Sporocyst of *Macroderoides typicus*.
- Fig. 2. Cercaria of *Macroderoides typicus*.
- Fig. 3. Mature metacercaria of *Macroderoides typicus* from a tadpole.
- Fig. 4. Immature fluke from bullhead, 2-7 days after feeding.
- Fig. 5. Immature fluke from *Amia calva*, 7 days after feeding.
- Fig. 6. Immature fluke from *Amia calva*, 22 days after feeding.
- Fig. 7. Immature fluke from *Amia calva*, 49 days after feeding.
- Fig. 8. Adult *Macroderoides typicus* from *Amia calva*, 202 days after feeding.

TABLE I.—Average measurements of cercariae of *Macroderoides typicus*.

Parts measured	Number of measurements	Average in mm.
Body length	34	0.235 $\pm$ 0.0142*
Body width	29	0.086 $\pm$ 0.0077
Tail length	21	0.141 $\pm$ 0.0075
Oral sucker width	31	0.039 $\pm$ 0.0017
Acetabulum diameter	33	0.043 $\pm$ 0.0026

* In the present paper the standard deviation is used in the table of measurements of the series of individuals to show the expected deviation from the mean.

intestinal ceca could be easily seen and traced to the posterior end. These three cercariae probably belong to related species.

Cercariae of *M. typicus* were placed with many different species of animals in an attempt to find the second intermediate hosts. It was found that there was penetration, at least, into several of the animals exposed, i.e., minnows and other small fish, snail species, *Physella mangellicustris* and *P. parkerii*, bullheads, salamander larvae, and tadpoles. In the minnows the cercariae failed to encyst. In the snails there was encystment but the metacercariae died in 2–4 days. In small bullheads and salamander larvae there was encystment and normal metacercariae were found several days after exposure to the cercariae but the extent of life in these hosts was not determined. With the tadpoles the cercariae penetrated and encysted in enormous numbers and since these animals were easy to keep in the laboratory they were selected for use in the later experiments. The tadpole is evidently a normal host because no death of the metacercariae was observed over a period of three months after exposure.

During the exposure to the cercariae the tadpoles showed some discomfort, became quite active and in some cases even jumped out of the containers. When the tadpoles were exposed to large numbers of cercariae the active penetration caused the appearance of haemorrhagic spots in the skin.

After the exposure of the tadpoles to the cercariae bits of the tails were removed and examined at intervals to follow the development and to determine the intensity of the infections. Soon after encystment the enlarged, oval excretory bladder was found to contain the concretions seen in the body of the cercaria. The removal of these concretions from the body tissues had a clearing effect so that the intestinal ceca could be seen extending into the posterior end. At the same time the stylet glands began to disintegrate. It was found that a maximum development was reached in about 8–10 days after encystment, by which time the metacercaria (fig. 3) has increased in size to about 0.3×0.1 mm. At this stage the anlage of the ovary can be seen as a rounded

mass of cells at the posterior border of the acetabulum and the developing testes are visible as obliquely placed, ovoid bodies on opposite sides of the excretory bladder. There is very little change in the relationships of the other organs. The metacercariae were studied over a period of three months after encystment but no further changes were observed. As the cysts grew older the wall became thicker and more opaque, due partially to the deposition of small quantities of pigment.

The metacercariae from the tadpoles were fed to a wide variety of animals, which included members of all five classes of vertebrates. The first positive indications were obtained when four small immature trematodes were recovered from the small intestine of a bullhead one week after it had been fed bits of experimentally infected tadpoles. The other fish that were examined later in the same experiment were negative. The experiment was repeated several times and it was found that excystment took place in the intestine of bullheads, and that some of the immature flukes would remain for 4-7 days, but after that time none were present. These immature flukes (fig. 4) show some development and measure about 0.43×0.09 mm. One of the first changes that takes place is the expulsion of the concretions from the excretory bladder. This leaves a long narrow bladder, shaped like that found in the cercaria, extending forward between the testes.

From the above experiments it was concluded that the species under consideration was a parasite of fish but that the bullhead was an abnormal host. Other species of fish gave negative results until *Amia calva* was tried. Seventeen small specimens of *Amia calva* obtained from a biological supply house and four of them were examined as controls for trematodes. Two were negative but in the others 35 trematodes were found in the posterior part of the intestine, which were identified as *Macroderoides typicus*. On account of this previous infection seven of the remaining animals were kept as further controls. To each of the six that remained one experimentally infected tadpole was fed. Individuals from both groups were examined at intervals to obtain comparisons of the natural and experimental infections and to trace the development. The results of these examinations are shown in table 2. In the eleven controls there was a total of 89 specimens of *M. typicus*, or an average of about eight in each animal. In the experimental animals a total of 3,700 specimens were recovered with an average of about 617 in each animal. It was noticed that the experimental animals seemed to have a more ravenous appetite and some difficulty was experienced in keeping them alive. In fish that were heavily infected the section of the intestine where the parasites were located was inflamed. The trematodes from the natural infection were easily separated from those experimentally induced in

TABLE 2.—*Individual records of experimental and control specimens of Amia calva examined for trematode infection.*

Number	Controls		Number	Experimental	
	Days in laboratory	<i>M. typicus</i> recovered		Days after feeding	<i>M. typicus</i> recovered
1	7	0	1	7	900 (estimated)
2	7	8	2	22	1392 (counted)
3	7	27	3*	23	260 (estimated)
4	7	0	4*	28	615 (estimated)
5*	10	2	5	49	500 (estimated)
6	18	7	6*	202	33 (counted)
7	18	0			
8	45	1			
9	45	14			
10	70	2			
11	294	28			
Totals		89			3700
Average per fish....		8			617

* Died.

the first four examinations, i.e., up to 28 days after feeding. There was a marked size difference and the presence of large numbers of eggs in the uterus made it easy to distinguish the older trematodes from those of the experimental infection. In the fish examined seven weeks after feeding the separation was more difficult, but it could still be made on a basis of size and the number of eggs in the uterus.

The first examination of an experimental animal was made seven days after feeding. The posterior portion of the small intestine was almost filled with immature trematodes, it being conservatively estimated that there were at least 900 present. When these flukes were compared with those recovered from the experimentally infected bullheads it was evident that considerable change had taken place. They (fig. 5) measure about 0.55 mm. in length, the vitellaria are faintly visible and the gonads have increased in size. The most striking change is found in the relationship of the excretory bladder to the neighboring organs. In the lengthening of the posterior end of the body the bladder remains more or less stationary and at this stage does not pass between the testes. It reaches only to the posterior border of the posterior testes and the main collecting tubes pass between the testes, then branch into anterior and posterior tubules at a level just posterior to the acetabulum. The relationship of the other organs remains unchanged.

On the 22nd day after feeding a second experimental animal was examined and the infected area of the intestine was again found to be almost filled with immature flukes. By actual count, 1,392 were recovered. In examining several of the living trematodes, flattened

under a coverslip, it was noticed that there was considerable variation in the rate of development. Some seem to have made no change from those found in the animal examined after 7 days, while in the better developed trematodes (fig. 6), the vitellaria are easily seen, the developing cirrus curves around the acetabulum and opposite to it is a beginning of the uterus. The relationship of the other organs remains unaltered.

Within a week after this examination had been made, two of the experimental fish died and 260 and 615 immature trematodes, respectively, were recovered. In comparison with the trematodes recovered from the previous experimental animal these showed very little change, but it was noticed that the development was more uniform in the trematodes from the fish harboring the lighter infection. It was also noted that the average development of these trematodes was more advanced than those recovered from the more heavily infected animal. A few of the trematodes in the lightly infected animal had 1-3 newly produced eggs in the proximal portion of the uterus.

In another experimental animal, examined seven weeks after feeding, approximately 500 immature flukes were recovered. These also show marked variation in the rate of development, with the smaller flukes showing a beginning of a cirrus and the larger having 1-10 eggs in the uterus. The most mature trematodes measure about 0.8 mm. in length and it was from these (fig. 7) that the first positive identification was made of the developing trematodes of the experimentally induced infections. At this stage, however, it was still possible to distinguish the trematodes of the experimental infection and by comparing the immature forms with the adults of the natural infection the identification was easily confirmed.

The marked variation, observed in the trematodes obtained from fish that were heavily infected, was probably due to crowding. The fish used in the experiments were small, the number of metacercariae introduced was enormous and the trematodes were confined to a small portion of the host's intestine. At first, naturally, there was no marked variation but as the experiment progressed the variation in the rate of development became more and more pronounced. This variation in the rate of development and the presence of such large numbers of trematodes in the first seven weeks is of particular interest, because the remaining experimental animal was accidentally killed 202 days after feeding and only 33 flukes were recovered. They were all mature and no separation of the experimental and natural infections could be made. This enormous loss of parasites was probably also due to crowding. A somewhat similar situation has been reported by McCoy (1930), in which a study was made of the rate of loss of *Hamacreadium gulella* in snappers that had been heavily infected experimentally.

The adult flukes (fig. 8) that were obtained from the last experimental animal were examined, partially flattened, in the living condition before fixation and were identified as *Macroderoides typicus*. In comparing this living and preserved material with that described by Winfield (1929) it was found that nothing of importance could be added to his description. It was found that the flukes were, on the average, larger than those described by Winfield, but this can easily be accounted for in the degree of flattening. He reported finding an equal number of amphitypes. In examining 20 random specimens from the experimental infection it was found that 14 trematodes had the cirrus, ovary and posterior testes on the right side, and the anterior testes on the left. In six specimens the reverse arrangement was found. Winfield figures the latter type while figure 8 in the present paper shows the former.

This species, *Macroderoides typicus*, was first described by Winfield (1929) as *Plesiocreadium typicum* from the intestine of *Amia calva*, taken in the Douglas Lake region. The newly created genus was placed in a new subfamily Plesiocreadinae, in the family Allocreadiidae. Van Cleave and Mueller (1932) pointed out the similarity of this species to those included in the genus *Macroderoides* (Pearse, 1924) and placed the species in this genus, thus transferring the species from Allocreadiidae to the Plagiorchiidae. Later Van Cleave and Mueller (1934) transferred the genus to the Allocreadiidae on the basis of the bag-like bladder. The position of this genus will be discussed later in the present paper.

THE LIFE CYCLE OF *Alloglossidium corti* (LAMONT)

The cercariae of *Alloglossidium corti* were found to penetrate into and encyst in dragonfly and mayfly nymphs. After the metacercariae fed to bullheads, adult flukes, identified as *Alloglossidium corti* (Lamont, 1921), were recovered from the small intestine. Crayfish, dragonfly and mayfly nymphs from the area of Munro Lake, where the cercariae were so abundant, were examined and natural infections of the metacercariae of this species were found in all three forms. Other small arthropods probably serve also as second intermediate hosts because small bullheads, 40 mm. or less in length, from this area had developing trematodes of this species in the intestine. These fish appeared to be too small to have fed on the arthropods that were found to be naturally infected.

McCoy (1928) described as *Plagiorchis ameiurensis* a trematode from the intestine of the bullhead, *Ameiurus nebulosus*, and experimentally demonstrated its life cycle. The cercariae were found in *H. trivolvis* and penetrated into and encysted in dragonfly nymphs and crayfish.

The adult flukes developed in the intestine of bullheads that had been fed with these metacercariae. Naturally infected crayfish were also found. Mueller (1930) pointed out that *P. ameiurensis* is a synonym of *P. corti* (Lamont, 1921). Simer (1929) had described this species as *Alloglossidium kenti* and gave it as the type species of the new genus in the family Allocreadiidae. Van Cleave and Mueller (1934) pointed out the identity of the forms described by Lamont and Simer but retained the genus *Alloglossidium*, removing the species from the genus *Plagiorchis* because of the presence of an I-shaped excretory bladder.

After the adults of the present experiments had been identified as this same species, the cercaria was compared with the description given by McCoy and it was found to correspond so exactly that nothing need be added to his description. The present paper adds another first intermediate host, *H. campanulata*, and demonstrates that dragonfly and mayfly nymphs serve as natural intermediate hosts.

DISCUSSION

It has been shown, in the review of the identification of the two species, *Alloglossidium corti* and *Macroderoides typicus*, whose life cycles are discussed in the present paper, that previous authors have had varied opinions regarding their systematic position. They have been transferred back and forth between the families Plagiorchidae and Allocreadiidae. In most adult structures they resemble the Plagiorchidae, but the form of their excretory bladders is like that of the Allocreadiidae. Since their life cycles have been shown to be typical of the family Plagiorchidae their position in this group seems now to be definitely established. The principal difficulty in placing the genera, to which these species belong, in their proper positions, heretofore, has been the presence of a bag-shaped excretory bladder, which is a character used to define the Allocreadiidae. In reviewing the literature on the excretory system of adults and cercariae of the members of the family Plagiorchidae, it was found that in different genera of this group transitional stages were present, from the Y-shaped bladder of the type genus *Plagiorchis* to the bag-shaped bladder of *Macroderoides*. This series illustrating the transition in bladder form is shown in the diagrams of Plate II. The genital pore, gonads and intestinal ceca are also shown in the diagrams to indicate their relationship to the bladder.

The members of the genus *Plagiorchis* (fig. 9), in both cercariae and adults, (Nöller and Ullrich, 1927; Tanabe, 1922) have a Y-shaped bladder with the main excretory tubules attached at the anterior tips of the arms. In the genus *Opisthioglyphe* (fig. 10) the cercariae, in respect to bladder structure, are similar to those of the genus *Plagiorchis*

(Joyeux and Baer, 1927; Sinitzin, 1905). The bladder in the adult also is Y-shaped but the arms are shorter than those seen in the members of the genus *Plagiorchis*. The next step in the alteration of bladder form is seen in the genus *Tremiorchis* (fig. 11) (Mehra, 1926), in which the bladder arms are found to be still more reduced. In the genus *Brachycoelium* (fig. 12) the excretory bladder of the adult is a long tubular organ with small pockets at the anterior end representing only vestiges of the arms of the Y-shaped bladder. This character of the bladder has been seen by the writer in living specimens of *B. hospitale* collected in the Douglas Lake region. The bladder in the cercaria and adult of *Alloglossidium corti* (fig. 13) (McCoy, 1928) is a long I-shaped organ with a slight expansion at the anterior end where the main excretory tubules merge with it. In the adult this bladder passes between the testes and extends to the posterior border of the ovary. The life cycle of *Macroderoides typicus* (fig. 14) is reported in the present paper and it has been shown that the cercaria of this form has the same type of excretory bladder as in the previous species. In the development of the adult, however, the bladder does not retain its original relationship, but remains as a short, bag-shaped organ that extends only to the posterior testis. This transition in the bladder form within the family Plagiorchidae indicates that the type of bladder alone cannot be used as a character of the family. There is in fact such a wide variation within the group that the shape of the excretory bladder must be considered only of secondary importance. A characteristic that is constant within this family, as far as known, is that the life history includes Xiphidocercariae which develop in sporocysts. The course of development and the structure of the larval stages are, therefore, of primary importance in distinguishing members of the Plagiorchidae from other families of trematodes.

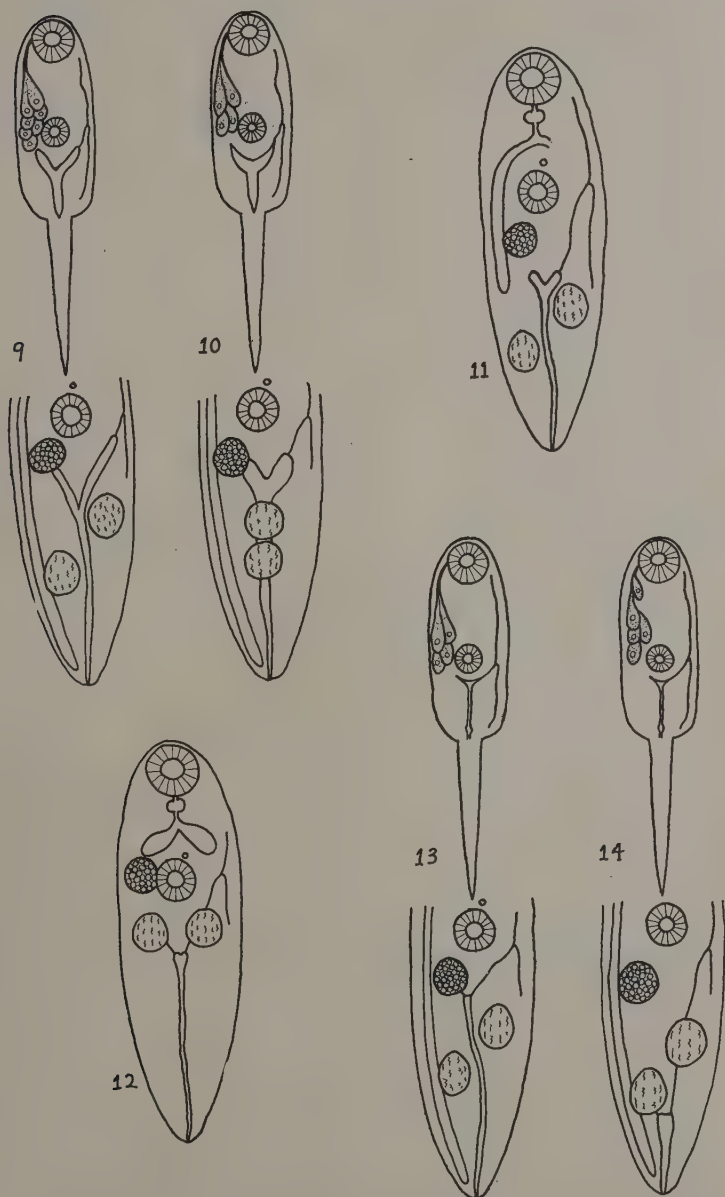
CONCLUSIONS

1. It has been demonstrated that a Xiphidocercaria is the larval stage of *Macroderoides typicus*. This cercaria develops in small rounded sporocysts in *Heliosoma trivolvis* and *H. campanulata*, penetrates into tadpoles and the adult develops in the intestine of *Amia calva*.

PLATE II.

Diagrams showing some of the changes in the shape of the excretory bladders in the members of the Plagiorchidae.

- Fig. 9. Type of the genus *Plagiorchis*, adult and cercaria.
- Fig. 10. Type of the genus *Opisthoglyphe*, adult and cercaria.
- Fig. 11. Type of the genus *Tremiorchis*, cercaria not known.
- Fig. 12. Type of the genus *Brachycoelium*, cercaria not known.
- Fig. 13. Cercaria and adult of *Alloglossidium corti*.
- Fig. 14. Cercaria and adult of *Macroderoides typicus*.



2. *H. campanulata* has been shown to be a new first intermediate host for *Alloglossidium corti* and mayfly and dragonfly nymphs were demonstrated to serve as natural second intermediate hosts.
3. Within the family Plagiorchidae, it has been shown that there is a transition in bladder form from the Y-shape of the genus *Plagiorchis* to the bag-shape of *Macroderoides*. This indicates that the shape of the excretory bladder should be used only as a secondary character within this family.
4. Since the life cycles of species in the genera *Alloglossidium* and *Macroderoides* have been shown to be typical of the Plagiorchidae, they are transferred from the Allocreadiidae to the Plagiorchidae.

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THE MORPHOLOGY AND DEVELOPMENT OF THE INFECTIVE LARVA OF *CYLICODONTOPHORUS ULTRAJECTINUS* (IHLE)

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With very few exceptions, comparatively little is known in regard to the morphology and development of the infective larvae of the numerous species of cyclostomes parasitic in horses. Poluszynski (1930) concluded, as a result of studies on the infective larvae of specifically unidentified cyclostomes, that the third-stage larvae of species of this group have, as a rule, intestines composed of 8, 16 or 20 cells. Whether infective cyclostome larvae possessing the same number of intestinal cells can be differentiated as to species on the basis of other constant characters, and whether these larvae can be distinguished from those of species of *Strongylus* and other genera of equine Strongylinae, can be determined only as detailed descriptions and figures of the third-stage larvae of the various species concerned become available. A knowledge of the morphology of cyclostome larvae is also a prerequisite to life-history studies.

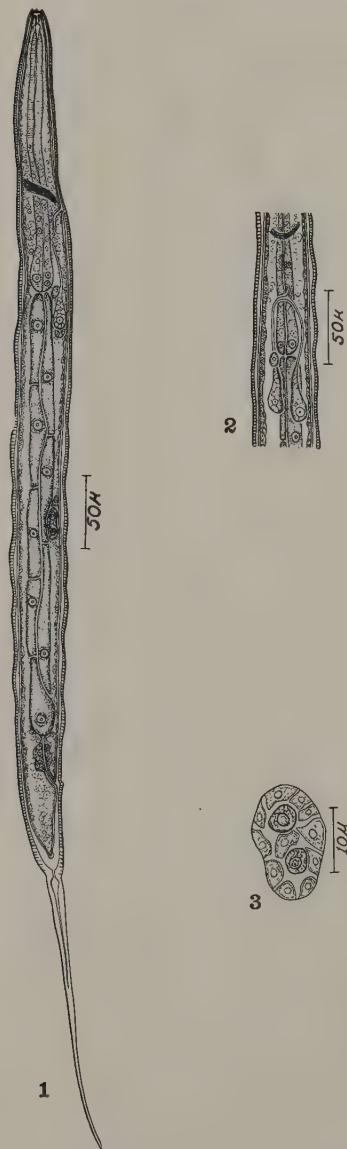
The third-stage larvae, upon which the description given in this paper is based, developed from eggs removed from individual females of *Cylicodontophorus ultrajectinus* cultured in water. Schwartz (1924) has reported that infective larvae of *Bustomum phlebotomum* obtained from water cultures invariably retained both the first and second sheaths. The larvae of *C. ultrajectinus* cultured by the writer also failed to exsheath following the first ecdysis and the infective larvae were enclosed in 2 sheaths. The second sheath only, which is retained by most third-stage strongyle larvae under all cultural conditions, is described in this paper.

MORPHOLOGY OF THE PREPARASITIC THIRD-STAGE LARVA OF *C. ultrajectinus*

Shape and size.—Body fusiform; tail short, tapering, usually slightly constricted a short distance anterior to the bluntly rounded tip. Ten larvae were from 387 μ to 566 μ long and from 19 μ to 32 μ wide.

Cuticle.—Transparent, of medium thickness; with transverse striae and prominent sinuous longitudinal lateral alae.

Sheath.—About three times thicker than cuticle; striae very prominent, the interstrial distance being at least twice that of the cuticle. Longitudinal alae present on lateral surfaces. Sheath conforming rather closely to contour of body, but markedly constricted just posterior to region occupied by tip of tail of fully extended larva; sheath extending posteriorly from constriction as a fine tapering hollow cuticular rod or tail 180 μ to 220 μ long (Fig. 1). Walls greatly thickened in region of constriction, lumen consequently narrowing suddenly to a fine canal; posteriorly lumen soon widening again with walls decreas-



EXPLANATION OF FIGURES

- Fig. 1. Ensheathed third-stage larva of *C. ultrajectinus*. Lateral view.
 Fig. 2. Ventral view of third-stage larva of *C. ultrajectinus* in region of excretory glands.
 Fig. 3. Genital primordium of third-stage larva of *C. ultrajectinus*.

ing in thickness, lumen then gradually decreasing in diameter with walls converging to posterior tip of sheath.

Alimentary canal.—Mouth opening surrounded by 6 minute papillae. Opening followed by a short narrow cuticularized canal leading to a minute inconspicuous vestibule. Anterior margins of vestibule in form of inverted V or U; lateral walls converging posteriorly in form of narrow V, embedded within esophageal tissue. Esophagus stronglyliform; 1 dorsal and 2 subventral nuclei of esophageal glands within esophageal bulb. Intestine of 8 cells, readily discernible in young larvae; boundaries becoming indistinct as reserve food material is exhausted. Rectum a narrow cuticularized canal leading to an inconspicuous anus. Rectal glands present.

Nervous system.—Nerve ring encircling esophagus slightly anterior to its equator. Chains of nerve cells, apparently 6 in number, passing anteriorly from nerve ring toward cephalic region. Following prominent nerve ganglia observed in stained specimens: 2 lateral ganglia extending posteriorly from nerve ring along esophagus about one-half distance to esophageal-intestinal union; a ventral ganglion extending posteriorly from nerve ring nearly to termination of esophagus; a small dorsal ganglion extending posterior to the nerve ring; 2 large caudal ganglia in rectal region. A ventral chain of nerve cells extending from near the ventral ganglion to a rectal commissure. Phasmids about 15μ to 18μ anterior to caudal tip, connecting to narrow canals passing internally and anteriorly toward caudal ganglia.

Excretory system.—Excretory pore ventral to esophagus, just posterior to nerve ring, connecting with excretory canal leading internally and posteriorly to transverse duct. Two subventral glands just posterior to esophageal-intestinal juncture, connecting by means of narrow canals with transverse duct (Fig. 2).

Genital primordium.—Genital primordium irregularly oval in lateral view; about 16μ long and 11μ wide; dorsoventral thickness about 5μ . Primordium transparent in living specimens; stained preparations showing 2 large centrally located germinal cells and 11 surrounding epithelial cells (Fig. 3). Primordium located ventral to posterior region of second ventral intestinal cell. In some specimens a spade-shaped "giant" cell appearing just anterior to genital primordium; another "giant" cell invariably found in body cavity near excretory glands.

The size relationships of 8 third-stage larvae are given in table 1.

TABLE 1.—Size relationships of 8 preparasitic third-stage larvae of *Cylicodontophorus ultrajectinus*. (All measurements in microns).

Specimen No.	1	2	3	4	5	6	7	8
Length of sheath	640	638	683	773	695	646	760	690
Length of larva	387	429	442	566	498	409	430	451
Width of larva at posterior end of esophagus	19	19	20	28	28	22	19	21
Width of sheath in the same region	25	27	27	35	35	28	25	29
Distance from anterior end to nerve ring	78	87	90	120	98	77	82	94
Distance from anterior end to excretory pore	91	96	103	139	..	95	93	103
Length of esophagus	165	168	165	176	173	155	155	167
Distance from posterior end of esophagus to genital primordium	87	101	115	155	122	104	111	115
Distance from genital primordium to anus	103	125	134	170	176	119	120	145
Length of tail of larva	32	35	28	49	27	34	33	28
Length of tail of sheath (Distance from point of constriction to posterior tip)	194	179	200	204	193	184	219	197

The third-stage larva of *C. ultrajectinus* is readily distinguishable from infective larvae of *Strongylus equinus*, *S. edentatus* and *S. vul-*

garis. It can be distinguished from the infective larva of *Poteriostomum ratzii*, recently described by the writer (1934), by the fact that the intestine of the larva of *C. ultrajectinus* is 8-celled, whereas that of the larva of *P. ratzii* is 16-celled. In other respects these 2 larvae are so similar that it is doubtful whether useful additional differential characters exist. A comparison of the size relationships of the larvae of the two species indicates that while the larva of *P. ratzii* is larger on the average, the measurements in all cases overlap.

PREPARASITIC DEVELOPMENT OF *C. ultrajectinus*

Eggs removed from individual female worms and cultured in water in small individual glass dishes hatched at room temperature (20° to 26° C.) in from 47 to 67 hours. Very few eggs had hatched when the cultures were examined after 50 hours, but 17 hours later all viable eggs had hatched. When the first-stage larvae hatched from the eggs, a small quantity of helminthologically sterile fecal extract was added to each culture. About 96 hours after the cultures were started, it was observed that many larvae had completed the first ecdysis and were in the second stage, although, as has been already mentioned, the first cuticle was not cast off. About 120 hours after the cultures were started, third-stage larvae were present, the second ecdysis, during which the larvae become completely stronglyliform in structure, having been completed by a majority of the larvae. The second cuticle was invariably retained by the third-stage larva as a sheath. An important phase of the development occurring during the second ecdysis is a reduction in the number of intestinal cells to 8. Young second-stage larvae have an intestine apparently consisting of 22 cells.

SUMMARY

Eggs of *C. ultrajectinus* cultured in water at room temperature hatched in from 47 to 67 hours.

When the first-stage larvae issued from the eggs helminthologically sterile fecal extract was added to the cultures. The first ecdysis was completed by many larvae about 96 hours after the cultures were started, but the first cuticle was not cast off. The failure of the larvae to cast off this sheath is attributed to the fact that they were cultured in a liquid medium.

About 120 hours after the cultures were started the second ecdysis was completed by a majority of the larvae. Third-stage larvae retained the second sheath also.

Third-stage stronglyliform larvae of *C. ultrajectinus* are from 387 μ to 565 μ long and have a short round-tipped tail. They have an intestine composed of 8 cells. They are enclosed in a thick sheath, varying in length from 640 μ to 773 μ , which extends from 180 μ to 220 μ beyond the posterior tip of the fully extended larva in the form of a tapering cuticular rod.

Infective larvae of *C. ultrajectinus* differ morphologically from the infective larvae of *Strongylus equinus*, *S. edentatus*, *S. vulgaris* and *Posteriostrongylus ratzii*.

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BALANTIDIUM COLI: QUANTITATIVE STUDIES IN EXPERIMENTAL INFECTIONS AND VARIATIONS IN INFECTIOUSNESS FOR RATS.*

ARNOLDO GABALDON

This work was undertaken in order to determine: (1) if differences exist in the susceptibility of the rat host when equal numbers of balantidia of a pure line strain are inoculated into different rats; (2) if significant relations exist between the infection density and (a) the weight of the large intestine plus contents and (b) the weight of the rat; (3) the relations between the inoculation density of balantidia and the total number and density of organisms present in the experimental host after intervals of three, six and nine days, and (4) possible variations in the infectiousness of various strains of *Balantidium* from the chimpanzee and pig for the rat.

1. *Material and methods.* Two strains of *Balantidium* from the chimpanzee were used (1) a Baltimore strain, a pure line isolated in this laboratory (Atchley, 1934) and (2) a Philadelphia strain, isolated from a chimpanzee in the Zoological Garden in Philadelphia.** Twenty-three strains from pigs killed in Baltimore were also used. They were taken from the colon of these animals a short time after death.

The rats were infected by injecting into the cecum 1 cc. of a suspension of balantidia following laparotomy. Except when otherwise stated, the balantidia used for these inoculations were taken the same day from the original hosts or from rats previously infected. All of the 296 animals used in these experiments were white rats; most of them were under 250 gms. They had been fed for one or two weeks previous to inoculation on a diet consisting of 85% carbohydrates (Schumaker, 1930).

Schumaker showed that a considerable variation exists in the number of balantidia in samples taken from different parts of the cecum, and since the parasites occur not only in this organ but also in the colon, a method was developed for estimating the number of balantidia in the entire large intestine. This method is a modification of the Stoll (1923) egg-counting method somewhat similar to that described by Atchley & Swezey (1934). It was as follows: (1) after killing the rat, the cecum, colon and rectum were taken out by severing the ileum

* From the Department of Protozoology of the Johns Hopkins University School of Hygiene and Public Health. The writer wishes to express his appreciation to Drs. Robert Hegner and Justin Andrews under whose direction this investigation was carried out.

** I am indebted to Dr. H. L. Ratcliffe for assistance in obtaining this strain.

near the cecum and the rectum near the anus. (2) The cecum, colon and rectum were opened in a petri dish and washed carefully with Ringer's solution in order to remove all the fecal material from these organs. (3) The fecal suspension was put in an Erlenmeyer-Stoll flask, which was filled with Ringer's solution until the 60 cc. mark was reached; then beads were added. The flasks were put in the incubator at 37° C. for from three to five hours, and shaken frequently to disintegrate the fecal material. (4) Afterwards, the liquid was well shaken, and 0.075 cc. taken up with a Stoll pipette. This quantity was put on a slide 50 by 70 mm.; one small drop of a solution of nine parts of 5% formalin and one of Bouin's liquid was added to kill the protozoa, and a cover-glass 22 by 30 mm. was carefully placed over it. (5) This preparation was then examined under the microscope with the 16 mm. objective and 5 ocular, and all organisms counted. Two preparations of each sample were counted, the numbers added and the sum multiplied by 400. The result gave the number of balantidia in the flask or from the whole large intestine.

In the search for balantidia, three samples whose weight totalled from 20 to 30 mgms. were taken from different parts of the cecum. They were diluted with normal saline solution and one preparation was made from each; this was examined under low power without a cover glass. It was estimated that by examining this quantity of fecal material, very low infections, from 100 to 300 organisms in the entire intestinal contents could be detected.

2. *Relation between the duration of the infection and the total number of organisms found, the number inoculated remaining the same.* Atchley (1934) working with cultures found in one experiment that the balantidia reached a numerical maximum at the third day of the inoculation and that this peak continued for 8 days, at which time the number began to decrease. In other experiments he found, with few exceptions, that the peak was reached between the first and fourth day of the inoculation and that the decrease began from one to three days later. In our experiments, three groups of rats were killed on the third, sixth and ninth days respectively after inoculation with an average of 1043.8 balantidia. Numerical studies of the infections were made during this period. The results of the counts are given in Table 1; these show that the infections reached their maximum on or before the third day and remained so for the remaining three or six days, since the averages obtained are not significantly different. In the classification of these rats, according to the number of balantidia found in the large intestine, a considerable individual variation was noted, since the percentages obtained were as follows: with less than 1000 organisms, 6%; between 1000 and 100,000, 73%; from 100,000

to 200,000, 13%; from 200,000 to 300,000, 6%; and more than 300,000, 2%. It is supposed that this variation must be due to the resolution of two opposing forces inherent to the parasite and to the host, tending to maintain and increase the number of organisms on the one hand and to lessen the number on the other. The tendency to multiply was probably uniform since the organisms all belonged to the same pure line. The variations must therefore have been due to the hosts, in which there may have been certain forces tending to inhibit reproduction and other forces tending to destroy the balantidia; to differences in the summation of these forces was due the numerical variation observed.

The number of trophozoites in the rat per individual trophozoite inoculated was estimated approximately by using the method of averages. Atchley (1934) found in his cultures in two instances, after six days of inoculation, 2000 and 1100 individuals respectively per trophozoite originally introduced. The figures obtained in the experiments reported here are lower (see Table 1). It is possible that Atchley's culture method provided a more favorable environment for *Balantidium* than does the large intestine of the rat. The number of ciliates per individual inoculated was very variable, a corollary of what was found regarding the magnitude of the infection. Here too, the averages obtained for the third, sixth and ninth day of the inoculation were not significantly different.

TABLE 1.—*Balantidia* in the large intestine of 73 rats inoculated each with 1043.8 trophozoites and killed at intervals of 3, 6 and 9 days.

Number of rats	Time after inoculation that the rats were killed	Number of balantidia in the large intestine of infected rats	Number of balantidia found per each <i>Balantidium</i> inoculated	
		Average	Average	Range
25	3 days	48200	46.2	1.4-311.4
26	6 days	37323	35.8	2.3-229.2
22	9 days	82654	79.2	1.6-268.2

3. *Relation between the weight of the large intestine plus contents and of the weight of the rat, to the infection density.* The utilization of a number of animals over a limited weight range (50-119 gms.) provided the opportunity of observing whether or not the infection density, that is, the number per centigram of large intestine plus contents, increased with the weight of the rat or of the large intestine. The data are given in table 2, where it appears that the actual population density remains at a level (the variations are not statistically signifi-

cant) irrespective of weight increments. It is to be noted that these results were obtained from young animals only.

TABLE 2.—*Relation of the number of balantidia found in the large intestine to weight of the large intestine and to the weight of 71 rats.*

Weight of rats (gms.)	Number of rats	Average weight of the large intestine and contents (cgms.)	Average number of balantidia in the large intestine	Average number of balantidia per centigram of large intestine and contents
50-59	13	240	26900	112.1
60-69	10	260	46000	176.9
70-79	14	306	65764	214.9
80-89	15	335	58080	173.4
90-99	12	328	62783	191.4
100-109	5	372	64240	172.7
110-119	2	355	78200	220.3

4. *Relation between inoculation density and (1) the total number of organisms found and (2) the infection density.* Although the total number of parasites inoculated was practically the same per rat, the number per centigram of large intestine and contents varied inversely with the weight of the large intestine plus contents. Thinking that the magnitude of the resulting infection might depend more upon the inoculation density than upon the total number of organisms inoculated, the total number found and the number of organisms found per centigram of large bowel and contents were classified according to the number of organisms inoculated per centigram of large intestine and contents. As shown in table 3 no significant correlation appeared between the inoculation density and the infection density.

TABLE 3.—*Relation of the inoculation density to the total number of balantidia found and to the infection density in 64 rats.*

Balantidia inoculated per cgm. of large intestine and contents	Number of rats	Total number of organisms found in large intestine		Number of organisms found per cgm. of large intestine and contents	
		Average	Range	Average	Range
2.0-2.9	16	48347	400-170700	140.7	9.1-456.7
3.0-3.9	16	79643	1400-325600	256.6	4.0-909.5
4.0-4.9	17	32288	800-280000	174.5	6.4-1144.5
5.0-5.9	7	49714	7600-256000	265.5	36.3-1383.8

5. *Variation in the infectiousness for the rat of strains of Balantidium from the chimpanzee and pig.* Nelson (1934) succeeded in infecting rats with balantidia from the chimpanzee. Atchley (1934) working with the same parasite inoculated it intracecally into white rats on an 85% carbohydrate diet and obtained an infection rate of 94.0%.

With this same strain, in our experiments, it was found that of 109 white rats used and killed between the third and ninth day after the inoculation of organisms, 96, or 88.1%, developed the infection. This strain was lost in the summer of 1934 after having been transferred from rat to rat for one year.

In the fall of 1934 another strain from a Philadelphia chimpanzee was isolated. Ten rats were inoculated the same day that the fecal sample was passed; only two of these animals were found to be positive when killed three days later. With these balantidia three more rats were inoculated, but only one was found to be infected when killed 72 hours later.

Fecal extract cultures (Atchley, 1934) made from the same chimpanzee were inoculated from the original sample. From these cultures three groups of three rats each, were injected. One of these groups was without ciliates when killed three days after the inoculation; one rat in each of the other two groups had the organisms when killed at the same time. With the parasites from one of these rats, three more animals were inoculated, but none became infected. With those of the other rat and those from the positive rat of the groups infected from the original ten, three more animals were inoculated; they were also without balantidia when killed, thus terminating the strain. The rats used in these experiments came from the same stock as those employed with the Baltimore strain. The technique used, the weight of the animals, the diet and environment were the same in the work with both strains.

Schumaker (1929) reported for the first time the colonization of the large intestine of the rat with *Balantidium coli* from the pig, and showed that a diet high in carbohydrates increased the probability of producing and maintaining the parasitism. Andrews (1930) infected rats with balantidia from man and pig.

In the experiments reported here the isolation of *Balantidium* strains from twenty-three pigs was tried. The number of organisms inoculated into each rat was not known, but was estimated to be at least 1000. Each sample from the pig was inoculated into three rats and the transfers from rat to rat were also made in each case to three animals. The interval between the transfers was three days, which is an appropriate time as was shown with the Baltimore strain of balantidia from the chimpanzee. Fourteen of these strains did not develop in the rats; of the other six the one that remained longest survived only four transfers. Three other strains were inoculated using different numbers of balantidia in order to see if there was a variation in the number of animals that became infected. From eight rats inoculated each with 10360 balantidia, seven were found with the parasites; from seventeen

inoculated each with 1041, only eight showed the ciliates, and from eleven inoculated each with 104, only one had the organisms. All these rats were killed three days after the infection. It is considered that if the animals infected with the smallest number of balantidia had developed the infection, it could be found by the method of examination used. It is seen then that the larger the number of parasites inoculated the more chance there is of obtaining an infection.

It is difficult to explain the failure to establish the Philadelphia strain of *Balantidium* from the chimpanzee, and the twenty-three strains from the pig, in rats. This failure seems to indicate that the infectiousness for the rat is not uniform and that the power of adaptation of the parasites to this host is different in various strains of the same species of organisms. It is probable that the reproductive forces and the resistance to antiparasitic influences of the same species of *Balantidium*, are variable according to the strains of organisms used, when submitted to a foreign environment. This may explain the fact that *Balantidium coli* infection so wide spread in the pig is relatively rare in humans, although the chances for infection are great.

SUMMARY AND CONCLUSIONS

Experimentally produced balantidial infections in two hundred ninety-six white rats were studied.

In young animals (less than 250 gms. weight) on an 85% carbohydrate diet, inoculated intracecally with the same number of balantidia from the chimpanzee and killed three, six and nine days later, the average number of organisms found in the large intestine were not significantly different, which shows that the infection reached its maximum on or before the third day of the inoculation and remained so during the succeeding period of 3 or 6 days. It was found that the magnitude of the infection and the average number of balantidia per individual trophozoite inoculated was subject to a great variation.

The numerical density of parasites, that is, the number of organisms found per centigram of large intestine and contents, was likewise variable and was not influenced by the weights of the hosts used.

The inoculation density, that is, the number of organisms inoculated per centigram of large intestine and contents, did not appear to affect markedly the magnitude of the infection nor the infection density.

Balantidium coli strains from the chimpanzee and pig showed a variation in their infectiousness for the rat. Of two strains from the chimpanzee, one produced an infection in 88% of the white rats inoculated, while the other did not survive in them. Of twenty-three strains from the pig, thirteen did not develop in rats, while the other nine did,

but for only a short time; the two that remained longest survived only four transfers.

The *Balantidium coli* strains studied in these experiments showed a great variability in their infectiousness for the rat and in the magnitude of the infection in this host. Little correlation was observed between the intensity of colonization and factors which might be expected to influence it.

The data presented in this study indicate that *Balantidium* in a foreign host varies enormously with respect to infectiousness and ability to multiply in the intestine. Variability of these types have often been observed in this genus and also in other genera of intestinal protozoa, but it seemed worth while to obtain an accurate measure of this variability. The variation mentioned above may explain the fact that *Balantidium coli* infection so wide-spread in the pig is relatively rare in humans, although the chances for infection are great.

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SOME MICROCHEMICAL OBSERVATIONS ON CILIATES OF THE GENERA *BALANTIDIUM* AND *NYCTOTHERUS*

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Results obtained by the reagent of Feulgen on nucleus and cytoplasm. Trophozoites of *Balantidium coli* from pigs and guinea pigs, *Nyctotherus cordiformis* from frogs, and *N. ovalis* from cockroaches were used. The nuclear reaction of Feulgen (1932), showing the presence of thymonucleic acid, was applied in 1930 to *Balantidium* by Lucas, who observed a very clear positive reaction of the macronucleus and a negative or very weak reaction of the micronucleus; she noted also that the macronucleus showed colorless vesicular areas, similar to those previously described by her after using alum cochineal.

Employing the original technique of Feulgen, my results confirm those of Lucas on *Balantidium*. The very weak positive reaction which sometimes is observable in the micronuclei shows the presence of a small amount of thymonucleic acid, and it is probable that negative results when they occur may be due to the presence of thymonucleic acid in such a small quantity that the resulting reaction remains below the limit of visibility.

In *Nyctotherus* the results obtained by the nuclear reaction of Feulgen are about the same as obtained in *Balantidium*. It was noted that the karyophore of *N. ovalis* shows no positive reaction.

I applied also to *Balantidium* and *Nyctotherus* the cytoplasmic reagent of Feulgen, which shows the presence of plasmalogen. The reaction is based on the fact that the plasmalogen in presence of sublimate divides, producing a substance of probable aldehydic nature, which is subsequently revealed by the reagent of Schiff. In *Balantidium* the reaction is very intense. If some drops of Schiff's reagent are poured on a preparation previously fixed in sublimate after 30 seconds the violet staining of the protoplasm will have begun, and reaches a maximum in one minute, instead of 15-20 minutes generally required for the reaction. This reaction demonstrates that in *Balantidium* the plasmalogen is diffuse through the whole protoplasm; both the endoplasm and the ectoplasm show its presence but in the ectoplasm the reaction is less intense. When there are food inclusions in the protoplasm, these may

¹ From the Department of Protozoology of the School of Hygiene and Public Health of the Johns Hopkins University. This work was made possible by aid from the International Health Division of the Rockefeller Foundation. The writer wishes to acknowledge his indebtedness to Dr. Robert Hegner and Dr. Justin Andrews for their suggestions.

or may not give the reaction, depending on their nature; if there is no reaction, they appear like clear areas in the protoplasm. The contractile vacuoles show no plasmalogen, appearing colorless inside the intensely violet-stained protoplasm. Since the contractile vacuoles are considered organs of respiration and excretion, this behavior indicates that the plasmalogen is a constituent of the protoplasm and not a product of metabolism to be eliminated without subsequent chemical modifications.

In *Nyctotherus* the results were about the same as in *Balantidium*.

During these studies I observed that on staining *Nyctotherus* from the frog with methyl green, a green coloration of the nucleus and an intense violet coloration of the protoplasm were obtained. The violet protoplasmic coloration is dependent on the presence of methyl violet, which is normally mixed with the methyl green. The phenomenon may reveal in the protoplasm of *Nyctotherus* the presence of a considerable quantity of a substance with basophilic character, diffused through the whole protoplasm of the ciliate. Since the distribution of this substance is identical with that of the plasmalogen, the question of the identity of these two substances arises. While the plasmalogen is soluble in alcohol, the basophilic substance on the contrary remains in the protoplasm even after a period of 48 hours in 95 per cent alcohol. By this experiment the plasmalogen and the basophilic substance are demonstrated to be different.

Results obtained by the reagent of Schiff after fixation in alcohol. Reichenow (1928) in his researches on the Feulgen reaction upon protozoa, observed that by immersing a preparation previously fixed in alcohol for 12-24 hours for one-half hour in the reagent of Schiff a selective violet coloration of volutin is obtained. By the same technique, but immersing in the reagent of Schiff from 12-18 hours longer, I observed in *Balantidium* that the macronucleus appears more or less intensely yellow showing variations of color from gold yellow to orange and brownish yellow. If the immersion in the reactive is prolonged for 3-4 days, new modifications of color take place in the macronucleus which becomes more brownish or reddish. Sometimes in the preparations showing yellow-stained macronuclei, when mounted in balsam, similar modifications of color of the macronuclei occur during the following days.

These phenomena are not easily explained. A first hypothesis would consider that the reagent of Schiff is generally yellowish in color; it would be possible to assume that the macronucleus of *Balantidium* would have some affinity with the reagent absorbing it like a stain. But this hypothesis may be eliminated by the following experiment: if the preparation be carefully washed in tap water to remove the excess of the reagent and repeatedly immersed in a solution of formaldehyde, no variations in the yellow color of the macronucleus are observable, except

that sometimes it becomes slightly more brown.' This behavior demonstrates that the yellow substance is not a simple reaction to the reagent of Schiff, because if so the formaldehyde would react with it turning it to a violet color.

Probably the phenomenon can be considered as dependent on successive complex chemical modifications of the nuclear substances in the presence of the reagent of Schiff with production of new colored substances. This would explain also the above noted differences of color tones because these would depend on the action of different qualitative and quantitative factors. It is known, for example, that basic fuchsin, which is commonly used for preparing the reagent of Schiff, is really a mixture in variable proportions of three different dyes: pararosanilin (triamino-triphenyl chloride), rosanilin (triamino-tolyl-diphenyl-methane chloride), and the new fuchsin (triamino-tritolyl-methane chloride). This factor may be of considerable importance in the determination of the color phases of the new compounds.

In any case these observations reveal a behavior of the reagent of Schiff which is different from that observed in both the true and the pseudo-reaction of Schiff. In fact in the true reaction of Schiff the final product is of violet color and in the pseudo-reaction it is of fuchsin-red color.

A new method of nuclear staining by neutral red. During these studies I had occasion to observe in *Balantidium* a particular behavior of neutral red with nuclear substances, which appears not to have been previously reported. Neutral red is generally regarded as a weak nuclear dye and is used especially for vital staining and for studying the Golgi apparatus in cells. The phenomenon I have observed in the *Balantidium* of pig and guinea pig consists of a selective nuclear coloration after an immersion in an alcoholic solution of neutral red followed by differentiation in a solution of sulphuric acid.

The following method was used:

(1) A few drops of 3 per cent solution of neutral red in absolute alcohol are poured on a dry smear of feces containing *Balantidium*.

(2) After 30 seconds the preparation is washed in tap water. Under the microscope the nucleus and the protoplasm of *Balantidium* are entirely colorless.

(3) The preparation is immersed for a few seconds in a 2 per cent solution of sulphuric acid. Observation under the microscope shows the nucleus selectively stained red.

By remembering that the neutral red, which acts like an indicator, shows chromatic variations depending on the pH, two explanations of the phenomenon are possible:

(a) The nuclear substances react with the neutral red in alcoholic solution to form colorless substances; subsequently the sulphuric acid

produces a division of these compounds with consequent precipitation of the color.

(b) The nuclear substances act like a catalyzer in a direct reaction between the neutral red and the sulphuric acid.

The first hypothesis seems more probable, because if the technique is modified by immersing first in the sulphuric acid solution and staining subsequently by the neutral red alcoholic solution, a diffuse coloration of the whole body of the *Balantidium*, instead of a selective nuclear coloration, is obtained.

Whatever is the essence of this phenomenon, it demonstrates the possibility of obtaining a very rapid intense nuclear coloration by the use of neutral red.

SUMMARY

1. *Balantidium* and *Nyctotherus* show a strong nuclear reaction of the macronuclei and a weak or negative reaction of the micronuclei.

2. The karyophore of *Nyctotherus ovalis* shows a negative nuclear reaction.

3. In *Balantidium* and *Nyctotherus* the plasma-reaction is very intense and rapid so that one minute is sufficient to produce it instead of 15–20 minutes normally required.

4. The plasmalogen is contained in both the endoplasm and the ectoplasm, but in less quantity in the latter.

5. The plasmalogen is absent from the contractile vacuoles. Therefore it is a constituent of the protoplasm and not a product of cellular metabolism to be eliminated without subsequent chemical modifications.

6. In the protoplasm of *Nyctotherus* was observed a substance which shows a strong affinity with methyl violet. There is no identity between this substance and the plasmalogen.

7. In *Balantidium*, prolonged treatment with the reagent of Schiff produces a yellowish nuclear coloration, which subsequently shows color variations probably in relation to modifications of the nuclear substances in the presence of the reagent.

8. A selective nuclear coloration is described, which is produced by using an alcoholic solution of neutral red followed by differentiation in a solution of sulphuric acid.

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TAXONOMY OF THE NEMATODE GENUS *SPIROXYS* (FAMILY SPIRURIDAE)*

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INTRODUCTION

In connection with a study of the life history and morphology of *Spiroxys contortus* (Rud.) during the years 1929-1932 inclusive I had the opportunity to compare my material collected from the stomachs of various species of North American turtles with specimens of *S. contortus* from Europe. At the same time all other members of the genus available to me were examined and redescribed, the taxonomy of the genus and its member species was revised and appropriate diagnoses were prepared.

HISTORICAL ACCOUNT

Rudolphi (1819: 242-243) was the first to describe a nematode belonging to the present genus *Spiroxys* under the name *Spiroptera contorta* from the stomach of *Emys orbicularis* (Linnaeus) (*Testudinis orbicularis*). He intimated that *Spiroptera contorta* might be synonymous with *Ascaris testudinis*, but stated (1809: 198-199) that he was unable to obtain the appendix by Braun to Schneider's "Naturgeschichte der Schildkröte" (1789) in which a description supporting this name was supposed to appear. Baylis and Lane (1920) also failed to find the reference and designated *Ascaris testudinis* as a *nomen nudum*. Schneider (1866: 125-126) established the genus *Spiroxys* to include only one species, *contorta* R. which he separated from the genus *Spiroptera* R. He failed to change the ending of *contorta* to *contortus* to make it agree in gender with *Spiroxys* with a masculine termination. Indeed, most of the subsequent authors of specific names for mem-

* Contribution from the Zoological Laboratory and the Biological Station of the University of Michigan.

I wish to express my sincere appreciation to Dr. George R. LaRue, Professor of Zoology and Director of the Biological Station of the University of Michigan for his suggestions during this study. For the loan of material thanks are due to the British Museum of Natural History, the University of Berlin Museum, the late Dr. N. A. Cobb of the U. S. Department of Agriculture, Dr. P. D. Harwood of Vanderbilt University, Dr. H. B. Ward, emeritus professor of zoology at the University of Illinois, and Dr. Percy B. Moore of the University of Pennsylvania for some of Leidy's material from the Philadelphia Academy of Natural Sciences. I am grateful to Dr. Werner Schröder of the University of Berlin for supplying me with living specimens of the European turtle, *Emys orbicularis*.

bers of the genus have likewise used feminine endings with this masculine generic name. Throughout the historical review the original spelling of specific names will be used but elsewhere in this paper, the correct endings will be given.

Seurat (1918) described a nematode from the stomach of an Algerian turtle, *Clemmys leprosa* (Schweigger) and identified it as *Spiroxys contortus* R. Baylis and Lane (1920) described in detail a nematode from the stomach of *Emys orbicularis* under the name of *Spiroxys contorta* R. They pointed out that Seurat's description differed in several fundamental particulars from their material. The exact relation of Seurat's material to other members in the genus will be considered below. Baylis and Lane (1920) described *Spiroxys gangetica* from the stomach of an Indian turtle *Trionyx gangeticus* Cuvier. Baylis and Daubney (1922) added a third species, *S. annulata* from the stomach of another Indian turtle, *Chitra indica* Gray. Morishita (1926) described *Spiroxys japonica* from the small intestines of three species of Japanese frogs, *Rana nigromaculata*, *japonica* and *rugosa*. Walton (1927: 146-147) reexamined Leidy's material from North American turtles and made Leidy's *Ascaris sulcata* Rudolphi and *Ascaris penita* Leidy synonymous with *S. contorta*. He also stated that *Physaloptera contorta* Leidy differed from *Spiroxys* and could not be a synonym of *S. contorta* as suggested by Baylis and Lane (1920). Walton (1927: 147-149) transferred Leidy's *Physaloptera constricta* which was taken from the stomach of *Natrix sipedon* (Linn.) (*Tropidonotus sipedon*) to the genus *Spiroxys*, retaining, however, the specific name. Karve (1928) described *S. torquata* from the stomach of an Indian turtle *Emyda granosa intermedius* Annandale. Cobb (1909) described *S. amydae* from the stomach of *Amyda ferox* (Schneider). Walton (1930) described *S. allegheniensis* from the intestine of *Cryptobranchus allegheniensis* (Daudin).

MATERIAL

Approximately 400 adult *S. contortus* were collected by me from North America turtles. I also had taken *S. contortus* from *Emys orbicularis* (Linn.) from Europe, and *S. amydae* from *Amyda spinifera* (Le Sueur) from Mississippi. Specimens of the following species were borrowed for study: *contortus*, from the British Museum, University of Berlin Museum, and Dr. P. D. Harwood; *amydae*, from the late Dr. N. A. Cobb and Dr. Harwood; *allegheniensis*, from Dr. H. B. Ward, and material identified by Walton as *contortus* and *constricta* from the Leidy collection in the Philadelphia Academy of Natural Sciences.

CLASSIFICATION

The genus *Spiroxys* has been variously placed, by Railliet and Henry (1916) in the Oxyuridae and by Seurat (1918) in the subfamily Camallaninae, family Heterakidae with the genus *Camallanus* Railliet and Henry (1915). Baylis and Lane (1920) created the new subfamily Spiroxyinae, family Gnathostomidae to include only *Spiroxys*. Chitwood and Wehr (1932) followed Baylis and Lane but added *Hartertia* to the Spiroxyinae. Yorke and Maplestone (1926: 302) incorporated the sub-family Spiroxyinae with the genera *Protospirura* Seurat (1914), *Hartertia* Seurat (1914) and *Spiroxys* in the family Spiruridae Oerley (1885). Absence of the cuticular head-bulbs and cervical sacs, characteristic of the Gnathostomes, in *Hartertia*, *Protospirura* and *Spiroxys* and the presence of a glandular esophagus in contrast to the muscular esophagus in the Gnathostomes serve as the principle reasons for retaining these genera in the family Spiruridae. Furthermore it has been proved that *Spiroxys* possesses gubernacula, so this supposed fundamental structural difference between *Spiroxys* and the other two genera disappears (Hedrick unpublished Ms.). *Protospirura* may be differentiated from the other two genera by its long vestibule, while *Hartertia* with two very unequal spicules is easily distinguished from *Spiroxys* with its equal to subequal spicules.

Since the life histories of certain species in the three genera are now known, a brief comparison of their life histories in relation to their classification is of interest. Theiler (1919) proved that the termite *Hodotermes pretoriensis* serves as the intermediate host for the bird inhabiting *Hartertia gallinarum*. Leuckart (1867: 112-116) demonstrated the meal worm *Tenebrio molitor* to be the intermediate host for *Protospirura muris* whose adult lives in mice. The essential difference between these two life histories and that of *Spiroxys* is the state of the development of the egg at deposition. The eggs of *Hartertia* and *Protospirura*, parasites of birds and mammals respectively, contain fully developed larvae when deposited but hatching does not take place until the eggs are eaten by the intermediate host, thus enabling the larvae to survive in a dry environment. Eggs of *Spiroxys* leave the definitive host in very early cleavage stages and soon perish unless they are in water where they incubate and hatch. The newly emerged larvae is free living and is attached to some object in the water with its sheath. It is quite apparent that this difference in degree of egg development and place of hatching is a correlative adaptation to the differences in the habits of life of the definitive hosts.

SPECIFIC CONSIDERATIONS

S. contortus. The nematodes collected by me from turtles were determined after careful comparison with material from England and

Germany to be *S. contortus*. Although these borrowed specimens were old and poorly fixed careful study revealed several fundamental structural characters such as cuticular collar with spines, colorless gubernaculum, and median genital papilla which had been overlooked in earlier studies. The finding of these structures removed the last doubt that my material was conspecific with the European *S. contortus*. This species has been redescribed by Hedrick (unpublished MS.).

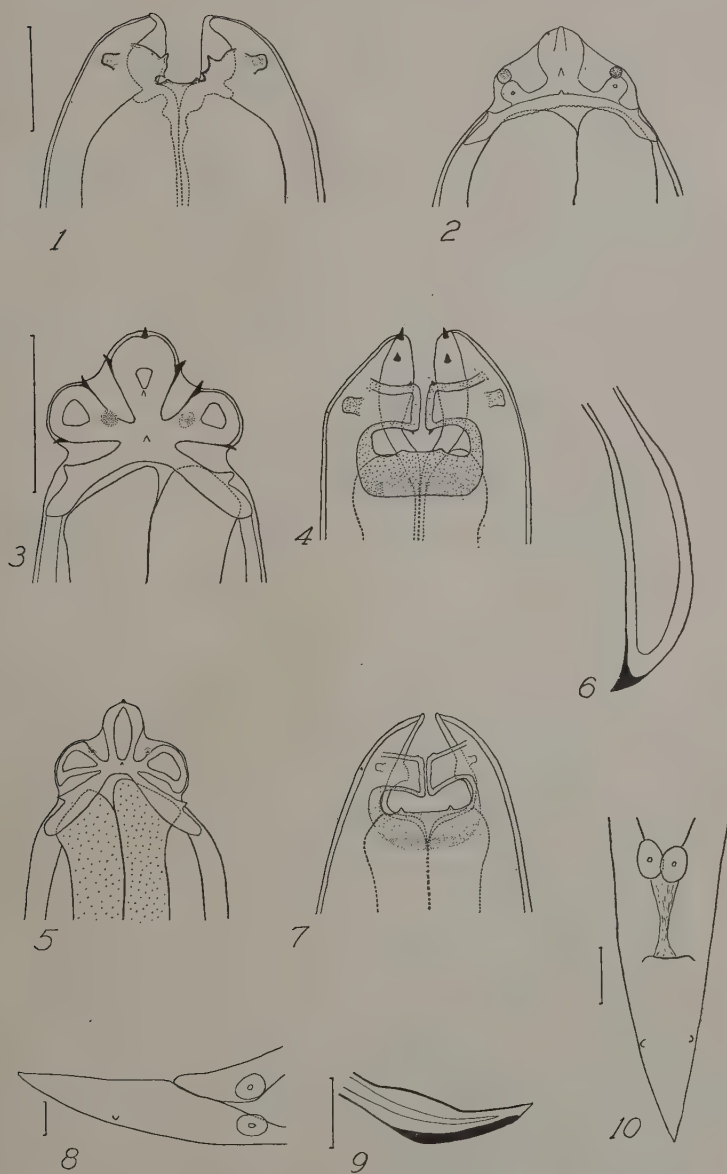
S. amydae. Examination of type specimens of *Spiroxys amydae* Cobb (1929) from the stomachs of *Amyda ferox* (Schneider) loaned to me by the late Dr. N. A. Cobb of the U. S. Department of Agriculture, specimens loaned by Dr. Harwood of Vanderbilt University and material collected by the author showed that these nematodes are very similar to the description of *S. gangeticus* Baylis and Lane (1920) except that adults of *S. amydae* are considerably smaller than those of *S. gangeticus*. A gubernaculum, cuticular collar with two dorsal and two ventral spines and one lateral spine, a median genital papilla in the male, and the two dorso-lateral caudal papillae on the tail of the female were also found in this species (Figs. 3, 4, 8, 9 and 10). The amphids reported for *S. amydae* by Cobb (1929) could not be detected in an *en face* view of a mounted head loaned by Dr. Cobb, although it was studied under an 1.8 mm. homogeneous immersion lens. My studies upon well preserved specimens of *S. amydae* show that the crenations near the posterior of the body reported for *S. amydae* by Cobb are not true characters but merely artifacts caused by poor preservation. The long narrow tail of the female *S. amydae* is quite distinctive.

Measurements of *S. amydae* in millimeters: lips, length, males, .061 to .064; females, .056 to .064; thickness, males, .109 to .119; females, .097 to .129. Length of body, males, 23 to 32; females, 26 to 37.

EXPLANATION OF PLATE

All the drawings were made with the aid of a camera lucida or a micro-projection apparatus; the details were filled in at the same magnification. The reference lines represent a length of one-tenth millimeter.

- Fig. 1. *S. allegheniensis*. Dorsal view of head with very small dorsal lobes of the lips.
 Fig. 2. *S. allegheniensis*. Lateral view of head, same magnification as Fig. 1.
 Fig. 3. *S. amydae*. Lateral view of head.
 Fig. 4. *S. amydae*. Dorsal view of head, same magnification as Fig. 3.
 Fig. 5. *S. constrictus*. Lateral view of head, same magnification as Fig. 3.
 Fig. 6. *S. constrictus*. Gubernaculum, lateral view, same magnification as Fig. 3.
 Fig. 7. *S. constrictus*. Dorsal view of head, same magnification as Fig. 3.
 Fig. 8. *S. amydae*. Posterior end of female, lateral view showing caudal papilla.
 Fig. 9. *S. amydae*. Gubernaculum and end of spicule, lateral view.
 Fig. 10. *S. amydae*. Tail of female, ventral view showing caudal papillae.



Diameter of body, males, .372 to .56; females, .38 to .59. Tail of males moderately long, .232 to .264 mm. long; tail of females long and narrow, .36 to .48 mm. long and .18 mm. wide at anus. Spicules robust, about .72 to 3.37 mm. long. Distance between cuticular striations 5 to 11 micra.

S. constrictus. Walton (1927) reexamined Leidy's material and allocated *Ascaris penita* Leidy (1886) and *Ascaris sulcata* Rudolphi (1819) Leidy (1887) to the genus *Spiroxys*, species *contortus*. He also transferred to this genus specimens that Leidy found in the stomach of *Natrix* and had named *Physaloptera constricta* Leidy (1856). After a careful study of Leidy's specimens of these three species, it is my opinion that all of these worms are conspecific and that they belong in the genus *Spiroxys* as Walton indicated, but to the species *constrictus*, not *contortus*. Of the three names used by Leidy, *constrictus* is the only one available because Rudolphi's species *A. sulcata* clearly belongs to the superfamily Ascaroidea. Rudolphi described the head of this species as distinct and provided with three large lips, "*Caput valvulis tribus majusculus instructum, discretum.*" In contradistinction to this, the same author described the head of *Spiroptera contorta*, a true *Spiroxys* and Spiruroid thus, "*Caput saepe tres tantum papillas in trifolii modum positas exhibet.*" *Constricta* Leidy (1856) has priority over *penita* Leidy (1886).

The worms of the existing Leidy collection examined by me are as follows: three male and three female specimens of *sulcata*, most of these immature, two male specimens of *constricta*, and fragments of female *penita*. The *sulcata* and *constricta* material was poorly preserved but, as intimated above, the *penita* material was much worse. Since these worms are of the same species and they are so few in number, the description and measurements below are a composite of the three, rather than of any one of the former species. These worms are slender and rather delicate. The most distinctive character is the posterior slanting of the dorsal and ventral lobes of the lips, that is the posterior margin of the dorsal and ventral lobes forms an acute angle with the longitudinal body axis instead of an approximate right angle, as is true in the other known species (Figs. 5 and 7). The cuticular support for the lips is delicate and the arch of the cuticular collar appears to be high. The spicules are very slender and have filiform pliable terminations. They are cross striated despite Walton's statement to the contrary in regard to *constrictus*. There is a definite gubernaculum which possesses less cuticular material than the gubernaculum of *contortus* (Fig. 6).

Measurements of *S. constrictus* in millimeters: lips, length, .048 to .087; thickness, .073 to .10; distance of cervical papillae from anterior

tip of head, .09 to 1.34; body diameter, .217 to .480; body length, 15 to 24; tail length, .17 to .25; spicule length, 1.82 to 2.45, and distance between striations, .003 to .006.

The fact that the specimens known to Leidy as *Physaloptera constricta* were found in snakes should not form a basis for the differentiation of this species, because *Natrix* is probably not the normal host for these nematodes. It seems to me that Walton correctly interpreted the situation in these words, "The Watersnakes living in a similar environmental condition, are perhaps not the original or normal host of this species . . . but certainly can act as satisfactory substitute hosts when accidentally infected." This is all the more plausible since it is probable that fish, tadpoles and frogs serve as the second intermediate hosts for *S. constrictus* as well as for *S. contortus*.

S. allegheniensis. The study of two male specimens of *S. allegheniensis* has revealed several fundamental structures overlooked by Walton (1930). The head appears very short and wide because the usual cervical constriction is absent. The dorsal and ventral lobes of the lips are greatly reduced, especially the cuticular elements supporting them (Figs. 1 and 2). The cuticular collar is narrow and broadly arched, the posterior margin of the arch irregular. The submedian papillae, lateral papillae, cervical spines, and lateral cuticular spines are prominent. The esophagus is stout and appears to be short, but is only relatively so because of its large diameter. The spicules are robust and cross-striated. The gubernaculum is reduced to a cuticular thickening of the curved spicule sheaths in the cloacal region. There is a definite median genital papilla. The cuticular striations are very fine.

Measurements in millimeters: lips, thickness, .14, length, .087 to .089; nerve ring to tip of head, .77; esophagus, length, 2.52, maximum diameter, .357; body length, 24 to 27; body diameter at nerve ring, .388; at end of esophagus, .775; at middle of body, .884; and at vesicular swelling, .667; tail length, .37, and spicule length, 1.7 to 2.0.

S. algericus n. sp. The nematode identified by Seurat (1918) as *Spiroxys contortus* from the stomach of *Clemmys leprosa*, an Algerian turtle, is said by Baylis and Lane (1920) to differ from their forms of *S. contortus* as follows:

- "(1) the presence of an internal tooth on *each* lobe of each lip;
- (2) the presence of a pair of large papillae on the middle lobe of each lip, instead of one small papilla;
- (3) the presence of an adanal, lateral pair of genital papillae in the male;
- (4) the presence of an accessory piece in the male."

Seurat's original description was carefully compared by me with specimens of *S. contortus* from the British and Berlin Museums and

in my opinion the interpretation of Baylis and Lane with respect to items one and two is correct. As a result of this comparison another difference can be stated. This is that the tail of the male in Seurat's form is 612 micra long as compared with the maximum length of 388 micra for the tail of an adult male *S. contortus*. It has been pointed out previously in this paper that the presence of a gubernaculum (accessory piece) in both groups is a point of similarity and not a difference. Reference has also been made by the author to the pair of adanal lateral genital papillae, indicating that this pair may be pair number 6, normal to the genus. The location of this pair number 6 is rather variable with respect to the anus, being sometimes posterior, at other times parallel, or anterior. As Seurat mentioned only 11 pairs in all, it is my opinion that this adanal lateral pair of genital papillae may be substituted for the normal pair, number 6.

Since Seurat is an experienced worker and a good observer, it must be assumed that his description is accurate. Therefore, the other differences, namely, the presence of an internal tooth on each lobe of each lip, the presence of a pair of large papillae on the middle lobe of each lip instead of one small papilla, and the long tail in the male instead of a tail of moderate length, constitute characters for a new species. These differences are so marked that a new name, *Spiroxys algericus* is proposed for this Seurat material, although it was impossible for me to study the specimens. However, if further study of this material should prove that it is really *S. contortus*, then *S. algericus* can be reduced to synonymy.

Measurements in millimeters for *S. algericus* from Seurat's description are: total length, 31 mm.; thickness, .60 mm.; nerve ring to anterior end, .47 mm.; cervical papillae to anterior end, .625 to .66 mm.; excretory pore to anterior end, .815 mm.; esophagus length, 2.74 mm.; tail length, .612 mm.; spicules equal, length, 3.63 mm., and gubernaculum length, .120 mm. All measurements are from male specimens.

DIAGNOSES

The finding of a gubernaculum in the males of the species of *Spiroxys* prevents the use of that structure as a character for the differentiation of *Spiroxys* from *Hartertia* and *Protospirura* and makes it necessary to amend the diagnosis given by Yorke and Maplestone (1926: 302) for the sub-family accordingly.

Sub-family Spiroxyinae Baylis and Lane (1920)

"Spiruridae: mouth with large distinctly trilobed lips; the cuticle of the inner surface is thickened and thrown into folds and tends to interlock with that of the outer lip; vestibule present without ring-like or spiral thickenings; cephalic and esophageal regions not ornamented with cuticular plaques." Gubernaculum present.

Type genus, *Spiroxys* Schneider (1866).

Other genera, *Hartertia* Seurat (1914) and *Protospirura* Seurat (1914).

Genus *SPIROXYS* Schneider (1866) emend.

Generic diagnosis. Spiroxyinae: head with two prominent trilobed lips; cuticle of median lobe thickened and usually projecting anteriorly to form blunt tooth; dorsal and ventral lobes tending to oppose those of opposite lip; each lip bearing two sub-median papillae and one small lateral papilla, rarely only two papillae on each lip, those on the median lobe. Cuticular collar usually with six spines, two dorsal, two ventral and one small spine on each lateral surface, rarely one dorsal and one ventral spine. Vestibule with cuticular lining, short, leading into elongate, club-shaped esophagus. Esophagus divided into two regions, anterior part short and muscular, gradually merging into longer glandular part. Male: caudal alae well developed, with preanal vesicular swelling; eleven pairs of papillae, four preanal and seven postanal; seventh and eighth pairs sessile, situated anterior and posterior to the anus respectively; one small median genital papilla anterior to the anus; spicules long, slender, cross-striated and equal to sub-equal; gubernaculum uncolored. Females: vulva near middle of the body; annulated vagina directed forward; two opposed, bi-pouched uterine branches in mature worm; two dorso-lateral caudal papillae on tail. Eggs pitted, deposited in one or two celled stage. Larvae, free-living, and sheathed. First intermediate host, Cyclops. Adults parasitic in stomach wall of turtles and snakes, and intestines of amphibia.

Type species: *Spiroxys contortus* (Rudolphi, 1819).

Synonyms:

Ascaris Rudolphi (1809: 193; 1819: 25 and 242) *e. p.*

Spiroptera Rudolphi (1819: 25 and 242) *e. p.*

Physaloptera Leidy (1856: 53) *e. p.*

Spiroxis Schneider (1866: 29) (misprint).

SPECIFIC DIAGNOSES

S. constrictus (Leidy, 1856) Walton (1927). Slender delicate *Spiroxys*. Males 17.5 to 23.5 mm. long, .21 to .326 mm. broad, females (only young specimens observed) 9.6 to 13.4 mm. long. Cuticular striations 3 to 6 micra apart. Tail short, length in males .171 to .187 mm., in females .25 mm. Spicules delicate, 1.82 to 2.45 mm. long, with filiform pliable terminations. Gubernaculum difficult to differentiate. Arch of cuticular collar high, cuticular supports for lips delicate. Dorsal and ventral lobes of lips slanting posteriorly, forming acute angles with median lobe. Lips, .048 to .087 mm. long and .073 to .10 mm. thick. Parasites in Leidy's collection from the stomachs of *Chrysemys picta* and *Natrix sipedon*.

Synonyms:

Physaloptera constricta Leidy (1856: 53).

Ascaris penita Leidy (1886: 312).

Ascaris sulcata Rudolphi (1819), Leidy (1887: 21-22).

Spiroxys contorta (Rud., 1819), Walton (1927: 146-147) *e. p.*

S. contortus (Rud., 1819), Schneider (1866). *Spiroxys* of medium size, males 19.5 to 26.6 mm. (av. 23.5 mm.) long, females 22.5 to 39.8 mm. (av. 27.7 mm.); maximum diameter of males .30 to .39 mm., of females .45 to .57 mm. Cuticular striae 5 to 6 micra apart. Tail length moderate, in males .233 to .388 mm., in females .18 to .45 mm. Spicules sharply pointed, 1.85 to 3.5 mm. long. Gubernaculum prominent, .108 to .110 mm. long. Arch of cuticular collar moderate, cuticular spines prominent, cuticular supports for lips moderate to heavy. Dorsal and ventral lobes of lips at right angles with median lobe. Lips of males .059 to .08 mm. long, .087 to .10 mm. thick; of females .07 to .122 mm. long, .09 to .13 mm. thick. Parasites in the stomachs of various species of turtles.

Synonyms:

Spiroptera contorta Rudolphi (1819: 242-243).

Spiroxys contorta (Rud., 1819), Schneider (1866: 125).

Spiroxys contorta Von Linstow (1909: 58).

Spiroxys contortus Railliet and Henry (1916: 114).

S. algericus n. sp. *Spiroxys* of moderate size, males 31 mm. long, and .60 mm. maximum diameter. Cuticular striations 7 micra apart. Spicules slender, equal, 3.63 mm. long. Lips three-lobed, each lobe bearing one small internal tooth, median or lateral lobes very large, each with pair of large papillae. Parasites in stomach wall of *Clemmys leprosa*, an Algerian turtle.

Synonym:

Spiroxys contortus (Rud., 1819), Schneider (1866), Seurat (1918: 23-25).

S. japonicus Morishita (1926). *Spiroxys* thick or stout, males 14 to 22 mm. long, females 18 to 31 mm.; maximum diameter males .46 to .64 mm., females .71 to .98 mm. Cuticular striations fine. Length of tail moderate, .33 to .37 mm. in males, .22 to .32 mm. in females. Spicules blunt, 2 to 2.2 mm. long. Lips of males .125 mm. long, .225 mm. thick, of females .15 mm. long, .36 mm. thick. Apparently only one spine on dorsal and ventral ends of cuticular collar. Cuticular annulations confined to anterior end of body. Parasites in duodenum of frogs, *Rana nigromaculata*, *japonica*, and *rugosa* in Japan (Morishita, 1926) and in *R. nigromaculata* in China (Hsü and Hoeppli, 1931).

S. torquatus Karve (1928). Large stout *Spiroxys*, males 19 to 36 mm. long, females 27 to 39 mm.; maximum diameter, males .277 to .66 mm., females .6 to .91 mm. Cuticular striations coarse, 17 to 18 micra distant. Tail moderately long, .21 to .3 mm. in males, .52 to .54 mm. in females. Spicules 2.25 mm. long, finely pointed. Arch of cuticular collar moderate to small. Dorsal and ventral lobes of lips at right angles with median lobe. Lips of males .075 to .09 mm. long and .112 to .15 mm. thick, of females .097 mm. long and .17 to .20 mm. thick. Parasites in stomach of *Emyda granosa intermedius* Annandale, an Indian turtle, and stomach of *Trionyx cartilagineus*, a Maylan turtle, Baylis (1933).

S. annulatus Baylis and Daubney (1922). *Spiroxys*, of medium size, males 20 to 25.4 mm. long, females 30 to 34 mm.; maximum diameter males .5 to .6 mm., females .8 to .85 mm. Cuticular striations very coarse, striae 25 micra apart. Tail very short, about .2 mm. long in both sexes. Spicules slender, sharply pointed, 1.6 to 2.3 mm. long. Arch of cuticular collar moderate. Dorsal and ventral lobes of lips at right angles with median lobe. Lips .06 mm. long and .016 mm. thick. Parasites in stomach of *Chitra indica*, an Indian turtle.

S. allegheniensis Walton (1930). *Spiroxys* stout, males 24 to 27 mm. long, maximum diameter .85 to .9 mm. Cuticular striations very fine. Tail length moderate, in male .36 to .37 mm. Spicules robust and short, 1.7 to 2 mm. long. Gubernaculum reduced. Cuticular collar delicate, posterior margin rough, only slightly arched. Dorsal and ventral lobes of lips extremely small, difficult to detect in dorsal view of head. Lips, .087 to .089 mm. long, .14 mm. thick. Males only described. Parasites in the small intestine of hellbender, *Cryptobranchus allegheniensis*.

S. amydae Cobb (1929). *Spiroxys* of moderate size, males 23 to 32 mm. long, females 26 to 37 mm.; diameter, males .372 to .56 mm., females .38 to .59 mm. Cuticular striations 5 to 11 micra. Tail of males moderately long, .232 to .264 mm.; of female long and narrow, .36 to .48 mm. long and .18 mm. wide at anus. Spicules robust, about 2.72 to 3.37 mm. long. Gubernaculum prominent. Cuticular collar moderately arched. Dorsal and ventral lobes of lips at right angles with median lobe. Each lip with special armature of six, fine sharp teeth, one on either edge of each lobe; cuticular tooth at apex of median lobe; sharp. Lips of males .061 to .064 mm. long, .109 to .110 mm. thick, of females .056 to .064 mm. long, .097 to .129 mm. thick. Parasites in stomachs of *Amyda ferox* (Schneider) and *A. spinifera* (Le Sueur).

S. gangeticus Baylis and Lane (1920). Large stout *Spiroxys*, males 39 to 43 mm. long, females 48 to 50.3 mm.; diameter, males .75 mm., females .85 to .95

mm. Cuticular striations moderately coarse, 8 to 17 micra apart. Tail long, .35 mm. in males; .65 mm. in females. Spicules robust 2.1 to 2.25 mm. long, ending in blunt points. Cuticular collar moderately arched. Dorsal and ventral lobes of lips at right angles with median lobe. Each lip carries special armature of six fine sharp teeth, one on either edge of each lobe; cuticular tooth at apex of median lobe sharp. Lips of males .14 to .16 mm. long and .11 to .13 mm. thick, of females .16 to .2 mm. long and .13 mm. thick. Parasites in stomachs of *Amyda gangeticus* (*Trionyx gangeticus*), an Indian turtle.

GEOGRAPHICAL DISTRIBUTION

The nine species of *Spiroxys* are distributed as follows: *annulatus* and *gangeticus*, India; *torquatus*, India (Karve, 1928) and Malay (Baylis, 1933); *japonicus*, Japan and China; *algericus*, Africa (Algeria); *contortus*, Europe and the United States of America, and *constrictus*, *alleghehiensis*, and *amydae*, the United States.

Although four species have been found in North America, their actual distribution is poorly known. *S. alleghehiensis* was taken from *Cryptobranchus alleghehiensis*, which is restricted to the streams of the Allegheny Mountains. This host is limited in range, but it seems likely that some member of the genus *Spiroxys* will be found in frogs and salamanders whose distribution in America is quite extensive. The forms now known as *S. constrictus* were probably collected from hosts found in the eastern part of the United States. No recent data are available in regard to the distribution of the two species above, as all of the worms known to be in the species *S. constrictus* were collected by Leidy before 1890 and the material called *S. alleghehiensis* was collected in 1909.

The distribution of *S. contortus* in America has not been considered until recently. For this reason, the known range for this species in the United States is Oklahoma and Texas (Harwood, 1931 and 1932) and Michigan, Louisiana, Wisconsin and Washington as determined by the writer. Since this species inhabits a variety of genera and species of turtles, its distribution in America is probably much more extensive than indicated above. Its rather wide distribution in Europe and America may indicate that it is an old form and that in ancient times the ancestors of the present hosts for *contortus* occupied the same geographical territory.

The known range for *S. amydae* is confined to the type locality, the Mississippi River, Texas as reported by Harwood (1932) and the state of Mississippi as determined by me. It is of interest to note that *Amyda gangeticus* (*Trionyx gangeticus*) in India is infected with *S. gangeticus*, which is very similar to *S. amydae*, differing only in size, the Indian worm being the larger.

Thus species of *Spiroxys* have been found in only a few isolated areas of the world. Nothing is known of their presence in Central or

South America, West Indian Islands, New Zealand, Australia, the East Indian Archipelago, in most of Asia, in most of Africa, and in the greater part of the United States. Because this parasite genus is known to inhabit many host species, in the groups Reptilia and Amphibia, and because of the possible discovery of a greater variety of hosts, it may be predicted that members of the genus *Spiroxys* will be found in nearly every part of the tropical and temperate regions of the earth.

SUMMARY

The genus *Spiroxys* has been reviewed and specimens of several species have been carefully studied. The following structures, that either had not been previously described or had been regarded as specific in rank, have been established as generic characters: gubernaculum, cuticular collar with dorsal and ventral spines and a small spine on each lateral surface, median genital papilla in the male, and the two dorso-lateral papillae on the tail of the female.

The nematodes, *Ascaris sulcata* R. Leidy and *Ascaris penita* Leidy, which Walton placed under *S. contortus*, have been shown to be conspecific with *S. constrictus* (Leidy, 1856), Walton (1927). This species has been redescribed.

The nematode described by Seurat (1918) as *S. contortus* has been found to be a new species and was named *S. algericus*.

Specific diagnoses for the nine recognized species of *Spiroxys* have been presented, some of them revised.

The present knowledge of the geographical distribution of the species of *Spiroxys* has been given.

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RESEARCH NOTES

A NEW SPECIES OF *BOVICOLA* (MALLOPHAGA)

A species of Mallophaga represented by several collections from the American elk or wapiti, *Cervus canadensis*, appears to be new and is described herewith.

Bovicola americanum n. sp.

Holotype, a female and 7 paratype females from *C. canadensis*, Yellowstone National Park, Wyoming, collected February, 1934, by Wm. Rush and the writer. The following specimens have also been examined and are paratypes: ninety-five females and 80 immature specimens from *C. canadensis*, Yellowstone Park, February, 1935, by Dr. C. B. Philip. Twelve females from *C. canadensis occidentalis* in the Olympic National Forest, Washington, April 19, 1933, by H. H. Stage. Thirty-five females and 29 immature specimens from *C. canadensis occidentalis* in the Olympic Mountains, Washington, March 12, 1935, by H. H. Stage. One immature specimen from *C. canadensis* in Cameron County, Pennsylvania, December 4, 1931, by H. S. Peters. The last 3 lots were loaned to the writer by the Bureau of Entomology and Plant Quarantine through the courtesy of H. S. Peters.

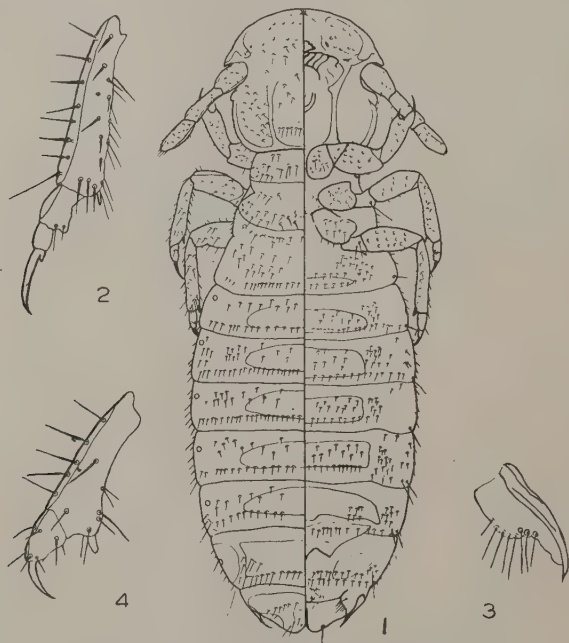


Fig. 1 *Bovicola americanum*, female.

Fig. 2. *B. americanum*, third tibia and tarsus.

Fig. 3. *B. americanum*, gonopod.

Fig. 4. *B. ovis*, third tibia and tarsus.

Holotype and paratypes deposited at the Rocky Mountain Laboratory at Hamilton, Montana. Paratypes also deposited at the United States National Museum, Bureau of Entomology and Plant Quarantine, and at Stanford University.

Female (Fig. 1) length 1.7 mm., head and thorax brown, abdomen light brown with darker cross bands. Head as broad as long. Forehead convex, broadly and evenly rounded. Trabecula not projecting from even curve of forehead. Very narrow pale interruption in the middle of the marginal band of forehead. Temples rounded. Posterior border of occiput slightly concave. Antennae three segmented, second segment longest. Numerous setae on all segments but no prominent spines. Mandibles dark. Thorax nearly as wide as head. First pair of legs smallest with well developed, pointed tibial spine opposing tarsal claw. Setae and a very small hyaline tibial spur opposing tarsal claws on tarsi 2 and 3 (fig. 2). Abdomen long ovate, wider than head. Tergal and sternal plates weakly sclerotized. Spiracles on segments 2 to 7. Sternal and tergal plates each with row of 26 to 34 and 32 to 40 fine setae respectively on posterior border on segments 2 to 6. Other setae irregularly placed on plates, more numerous laterally. Gonapophyses wide, with a marginal row of 7 to 9 setae on membrane. Membrane joins the lateral pointed chitinous blade near the tip (fig. 3).

Male unknown.

Although other biting lice from North American Cervidae have been placed in the genus *Tricholipeurus* Bedford (1929, 15th Annual Rept. Dir. Vet. Services, Union of S. Africa) by Peters (1930, Proc. Ent. Soc. Washington, 32: 76-79) this species definitely belongs to the genus *Bovicola* established by Ewing (1929, A Manual of External Parasites) for biting lice of Bovidae. Bedford (1932, 18th Rept. Dir. Vet. Services and Animal Industry, Union of S. Africa) included in it certain species from Equidae. Other members of the genus have been recorded in North America from domestic goats, sheep, cattle and horses.

The semicircular forehead separates this form from *B. bovis* of cattle, the elongate body distinguishes it from *B. caprae* of the goat. It differs from *B. equi* in being smaller and having the lateral blade of the gonopophyses projecting beyond the membranous portion. It is very similar to *B. ovis* from the sheep but may be readily separated by the shape of the third tibia. In *B. americanum* the tibia (fig. 2) gradually expands distally to twice the width of the proximal portion, whereas in *B. ovis* the tibia widens abruptly at the distal end forming a prominent lateral lobe on the inner side (fig. 4).—WM. L. JELLISON, Assistant Bacteriologist, United States Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana.

STUDIES ON *CLINOSTOMUM*. IV. NOTES ON THE PENETRATION AND GROWTH OF THE CERCARIA OF *CLINOSTOMUM MARGINATUM*

The contention that the yellow grubs (metacercariae of *Clinostomum*; Clinostomidae; Trematoda) of frogs and fish constitute two distinct and valid species has been supported by Cort (1913, Tr. Am. Mic. Soc., 32: 169-182), Hunter and Hunter (1934, Suppl. 23rd Ann. Rep. N. Y. St. Conserv. Dept., No. VIII, Rep. Biol. Surv. Raquette Watershed, 1933: 245-254), and Van Cleave and Mueller (1934, Roosevelt Wild Life Ann., 3: 161-334). Morphological data have been accumulated by the Hunters which, when analyzed statistically, show significant differences, thus indicating that the *C. marginatum* of fish and the *C. attenuatum* of frogs may be considered as separate species. Cross-feeding experiments by these authors on the definitive bird hosts, the great blue heron and the bittern respectively, furnish additional evidence to support this contention. The remaining "critical" experiment seemed to be the exposure of the piscine and amphibian hosts to the cercariae of a known species of *Clinostomum*. If this form developed metacercariae in both hosts it would cast doubt upon the previously performed cross-feeding experiments and the accumulated morphological data. If, on the other hand, metacercariae developed only in fish or amphibia is would definitely clinch the two species contention.

Experiment 34.41 began on October 23, 1934, when two common sunfish, *Eupomotis gibbosus*, and two tadpoles of *Rana clamitans* nearly ready to metamorphose, were exposed to cercariae identified as *Clinostomum marginatum*. The infected snail, *Helisoma antrosa* Conrad, came from a locality in New York state where one-half of the small-mouthed black bass examined were infected with this parasite. The fish and tadpoles used for the experiment came from a lake near Middletown, Connecticut, which has yielded neither infected amphibia nor fish. Thirty-nine control sunfish and 21 tadpoles of the same species (which are known from our records to be capable of harboring *Clinostomum*) were negative for this parasite. Four more of both fish and tadpoles were kept alive until the 20th week of the experiment when they were autopsied and found to be negative.

Both of the experimental tadpoles were negative, one being examined before metamorphosis and the other after. One experimental fish died and when found was too decomposed to use. The second is still alive (September 10, 1935) and appears heavily infected with *Clinostomum* eleven months after the initiation of the experiment. To date 14 specimens have been removed at different times and in different developmental stages. All were identified as *C. marginatum*.

Encysted metacercariae were first readily discernible during the 15th-16th weeks. At the 18th week three parasites were carefully removed from the gill cover. They were approximately 2.9 mm. in length and still possessed remnants of the pharynx, as did those of the 19th week (Cf. Figs. 3 and 4, Hopkins, 1933, Tr. Am. Mic. Soc., 52: 147-149). During the next week two worms encysted at the base of the dorsal fin were carefully removed. These were much further developed (probably due to a better location in the host?) and measured 3.7 and 3.95 mm. in length. The 21st week a morphologically fully developed metacercaria was removed from the fish. During the 25th week 2 metacercariae measuring 4.5 and 4.05 mm. in length were removed from the branchiostegal rays. A total of 14 viable metacercariae of *C. marginatum* have been removed up to and including the 45th week of the experiment. The host is still alive and still contains many *Clinostomum*.

It is apparent that under some conditions a period of about 20 weeks is necessary for the complete development of the metacercaria of *C. marginatum*. While a thermograph record of the temperature was not kept, periodic records of the water temperature showed variations between 21.7° and 25° C. It is obvious that the fish were in much warmer water than they would have been during a normal winter. This in turn suggests the probability that in nature many of these metacercariae attain their maximum development more slowly during the winter months. Whether or not this occurs is not definitely known. Other data gathered during the summer months suggest the possibility of a more rapid metacercarial development (Hunter and Hunter, 1934, J. Parasit., 20: 325). Krull, (1934, Proc. Helm. Soc. Wash., 1: 34-35) while recording penetration experiments in sunfish, fails to state the interval necessary for development of the metacercaria.

This experiment is of importance because it furnishes additional evidence of the validity of both *C. marginatum* and *C. attenuatum*. It may be considered as the critical experiment since previously recorded infection experiments have dealt solely with fish.—GEORGE W. HUNTER III, AND WANDA S. HUNTER, *Wesleyan University*. With the aid of a grant from the Denison Foundation for Biological Research at Wesleyan University.

A DEMONSTRATION OF VIABLE TRYPANOSOMES IN THE SEMINAL FLUID OF AN INFECTED GUINEA PIG

During some immunological studies of *Trypanosoma hippicum* Darling, it was found that a few of the infected male guinea pigs showed marked swelling of the genitalia. The scrotum was puffed and flabby to the touch and, upon aspiration, yielded several cubic centimeters of a clear watery transudate. Examination showed numerous active trypanosomes in the fluid, as well as several leucocytes per microscope field.

With the invasion of the genitalia by the organisms, it was thought that there might be a possibility of transmission of the disease during sexual intercourse. Although *Trypanosoma equiperdum* is normally transmitted in this way, it is generally conceived that there must be trypanosomal lesions on the genitals of the infected party. Because there are rarely, if ever, such lesions associated with *T. hippicum* infections, this method of transmission is given little credence.

A method described by Batelli (Comp. rend. Soc. physique et d'hist. de Genève., 39: 73, 1922) of inducing a seminal discharge in the guinea pig on stimulation by 30 volts of alternating electrical current on the head was used in this work. The ejaculation consists of spermatozoa and secretions from the various glands and vesicles of the genital system. As the ejaculum coagulates into an insoluble mass in a very few minutes after emission, microscopic examination for the trypanosomes was rendered difficult; nevertheless, examinations were made, but at no time were any organisms seen although motile spermatozoa were present. With this electrical ejaculator it was possible to collect in a sterile dish a single discharge from an infected guinea pig which was promptly inserted through a small incision into the peritoneal cavity of a rat. After a long incubation period (15 days), trypanosomes were seen in the peripheral blood. The rat died of the infection 24 days after inoculation.

In view of the observations of Darling (Trans. 15th Internat. Cong. Hyg. & Demog., Washington, Vol. V, part II: 619, 1912) that infection of mules is possible through intact vaginal mucosa; and the observation recorded here that viable trypanosomes may be carried in the seminal fluid ejaculated from an infected animal, it may be argued that infection of mares may follow breeding with infected stallions even when there are no lesions on the genitalia of the male.—HORACE F. SHARROCKS, Protozoological Laboratories, Department of Zoology, University of California, Berkeley, California.

GYRABASCUS BREVIGASTRUS, NEW GENUS, NEW SPECIES, A BAT
TREMATODE, WITH A NOTE ON DISTOMUM
MÉHELYI MÖDLINGER

Three examples of a new trematode were found by the writer, in 1933, during the examination of forty specimens of the little brown bat, *Myotis lucifugus*, taken from the attic of a house on the shore of Lake Pepin in the Mississippi River, at Stockholm, Wisconsin. While the new species, described below, may legitimately be placed in the family Lecithodendriidae Odhner, 1911 (Results of the Swedish Zoological Expedition to Egypt and the White Nile, 1901), it clearly does not belong in either of the existing subfamilies, Lecithodendriinae Looss, 1902 (Zool. Jahrb. Abt. f. Syst., 16: 8115), and Pleurogenetinae Looss, 1899, (Zool. Jahrb. Abt. f. Syst., 12: 521: 784), and therefore a new genus, *Gyrabascus*, and a corresponding new subfamily, Gyrabascinae, are erected for it.

Gyrabascus brevigastrus, n. g., n. sp. (Fig. 1)

Body pyriform, 0.84 to 0.97 mm. long by 0.43 to 0.55 mm. wide. Cuticula apparently aspinose. Cuticular glands inconspicuous, musculature reduced. Oral sucker 0.063 to 0.075 mm. long by 0.07 to 0.08 mm. wide. Pharynx 0.045 mm. long by 0.035 mm. wide. Oesophagus long. Intestinal ceca unusually short, ending near acetabulum. Ventral sucker 0.098 to 0.13 mm. long by 0.098 to 0.11 mm. wide, just pre-equatorial but far posterior to the oral sucker. Testes average 0.17 mm. long by 0.16 mm. wide, oval, postacetabular, in the posterior half of the body. Seminal vesicle 0.14 to 0.194 mm. long, situated immediately after the ventral sucker, sinuous, directed caudad. Cirrus sac and prostate mass absent. Genital pore posterior to genital pore and somewhat lateral in position. Ovary 0.114 mm. long by 0.142 mm. wide, anterior to testicular zone and behind ventral sucker, on opposite side of body to seminal vesicle. Seminal receptacle 0.055 to 0.062 mm. in diameter, located near mid-line of body, posterior to ovary. Vitellaria bilateral, consisting of compact masses of follicles, anterior border about half-way



Fig. 1. *Gyrabascus brevigastrus*, type, ventral view. Cam. lucida.

from intestinal fork to pharynx, posterior border overlapping ventral sucker. Uterus compact, filling posterior third of body, engulfing testes. Eggs 0.011 to 0.014 wide by 0.022 mm. long.

Host.—*Myotis lucifugus* (LeConte).

Location.—Small intestine.

Distribution.—United States (Stockholm, Wisconsin).

Type.—U. S. National Mus. Helm. Coll.; paratypes in author's collection.

DISCUSSION

Except for the equatorial instead of pre-equatorial position of the genital pore and the apparent absence of prostatic cells, the characters of this trematode place it in the family Lecithodendriidae. Absence of a definite cirrus sac excludes the present species from the subfamily Pleurogenetinae, and it also cannot be placed in the subfamily Lecithodendriinae since the genital pore is postacetabular and lateral instead of pre-acetabular.

For the reception of *brevigastrus* it is necessary to erect a new genus for which I suggest the name *Gyrabascus* (type species, *G. brevigastrus*). This genus is characterized by the following features: intestinal ceca very much reduced, in vicinity of acetabulum; ovary largely postacetabular; testes in posterior half of body; genital pore lateral, postacetabular; seminal vesicle sinuous, lying free in the parenchyma, not in a prostate mass; vitellaria in zone of ceca, consisting of two compact masses of follicles; body of uterus extending posterior to ovary, but engulfing testes.

The most closely related species appears to be *Distomum mēhelyi* Mödinger, 1930. The latter species agrees with *Gyrabascus brevigastrus* in that the genital pore is postacetabular, the seminal vesicle is coiled free in the parenchyma, and the ovary is pre-acetabular. In order to facilitate comparison, a diagnostic summary of Mödinger's species is presented.

Distomum mēhelyi Mödinger, 1930

Body elliptical, aspinose, 0.741 mm. long by 0.481 mm. broad, Oral sucker 0.28:0.25 mm. Intestinal ceca scarcely reaching posterior edge of oral sucker.

Testes oval, 0.296:0.199 mm., behind ventral sucker, at body middle. Ovary globular, 0.236 mm. in diameter. Vitellaria consisting of several large follicles, reaching as far as testes. Cirrus sac S-formed, extending left and posterior from near hind margin of ovary. Genital pore posterior to acetabulum. Uterus filling region behind testes. Eggs 0.022 mm. by 0.011 to 0.0129 mm. From small intestine of *Eptesicus serotinus*, Budapest, Hungary.

Since *Distomum mēhelyi* differs in generic characters from *G. brevigastrus*, I place *D. mēhelyi* in a new genus, *Ophiosacculus*, (type species, *O. mēhelyi*) which differs from *Gyrabascus* in these important respects: intestinal ceca rela-

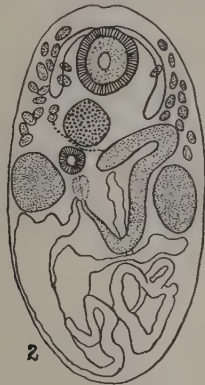


Fig. 2. *Ophiosacculus mēhelyi* (Modlinger), after Mödlinger.

tively long and slender and extend little past the oral sucker; ovary pre-acetabular, testes equatorial, genital pore postacetabular but nearly median; seminal vesicle sinuous but very long; vitellaria consisting of few, large follicles, with fields extending from oral sucker to testes; uterus posterior to testes.

Because of the fact that neither *Ophiosacculus* nor *Gyrabascus* may be placed in either of the existing subfamilies of the Lecithodendriidae, I propose for them a new subfamily, Gyrabascinae, (*Gyrabascus*, type genus) having the following diagnostic characteristics: genital pore postacetabular, lateral to nearly median; seminal vesicle sinuous, lying free in the body parenchyma, not surrounded by a definite prostate mass.

Should the seminal vesicle of *O. mēhelyi* be proven a cirrus pouch, then other provision would have to be made for this species.—RALPH W. MACY, *College of St. Thomas, St. Paul, Minn.*

BLOOD PARASITES OF BIRDS OF THE SYRACUSE (N. Y.) REGION

This study, which is still in progress, has so far included a total of 571 birds, belonging to 32 species. Of this number, 240 have been English sparrows, 120 starlings, 49 song sparrows, 31 slate-colored juncos, 18 purple grackles, 21 robins, 19 bank swallows, 16 cliff swallows, 16 white-throated sparrows, and 12 barn swallows. Other species, caught in smaller numbers, include the following: belted kingfisher, brown creeper, catbird, crow, chimney swift, chipping sparrows, humming bird, long-eared owl, great-horned owl, screech owl, pigeon, red-winged blackbird, sharp-shinned hawk, purple martin, ring-necked pheasant, Savannah sparrow, spotted sandpiper, tree sparrow, tree swallow, white-crowned sparrow, veery, and house wren. Most of these birds have been caught in traps, and the protected migratory species have been banded and later liberated. Methods of examination have involved the study of stained slides, cross-inoculation of the English sparrows (dividing the birds into pairs, and inoculating from A to B,

and the reverse) and subinoculation into fresh canaries from a number of the other birds (including 20 song sparrows, 12 bank swallows, 11 juncos, 6 robins, 5 cliff swallows, 4 grackles, 3 chimney swifts, 2 wrens, and 2 kingfishers). A number of the song sparrows have been caught more than once and it has therefore been possible to examine them at intervals.

The results of this study have shown that different species of birds differ widely in the parasites carried, and suggest that some of these differences may be due to differences in habit. Migratory birds are much more frequently hosts to blood protozoa than are non-migratory types (English sparrows and starlings). The song sparrow which summers in this latitude and winters farther south, appears to carry malarial infection much more frequently than the junco, which has a more northern range (13 out of 20 cases from which subinoculation was done in the former species have proved positive for malaria, as against 2 of 11 cases for the junco). Robins are very prone to infection with *P. vaughani*, as pointed out many years ago by Novy and MacNeal (1904, Amer. Med., 8: 932-934, but they also carry *P. praecox* almost as frequently, according to our experience. So far the former species of plasmodium has been found in no other species of bird, although a strain closely resembling it has been recently isolated from a song sparrow. There are important differences however: the latter strain lacks the refractile pigment often seen in *P. vaughani* and it produces a larger number of merozoites per segmenter. Further study and cross-inoculation will be necessary before it can be determined whether or not the malarial parasite of the song sparrow is a new species.

So far *P. praecox* (relictum), *cathemerium*, *circumflexum*, *elongatum* and *vaughani* have been found in birds locally caught, and also *P. nucleophilum*, n.sp. and *P. polare*, n.sp. (in addition to the not-yet-identified strain mentioned in the preceding paragraph). These last two species have been described elsewhere (Manwell, 1935, Amer. Jour. Trop. Med., 3: 265-283). Of all these types of malarial infection the two first-named seem the most common, and *circumflexum* comes next (but *vaughani* is very common in robins, as noted above). *P. polare* has so far been found only in three cliff swallows and *P. nucleophilum* in one catbird. *P. rouxi* has not yet been found at all. Numerous cases of *Toxoplasma* have occurred in English sparrows kept in the laboratory, and a few in canaries, but the method of transmission has not yet been determined. It is a moderately pathogenic parasite. Other parasites found have included *Hemoproteus*, *Leucocytozoon*, *trypanosomes*, *hemogregarines*, and *microfilaria*, but the last three have been very infrequent.

For diagnosing malarial infection subinoculation has proved vastly more reliable than microscopic examination, even when the latter is prolonged (half and hour per smear, or more). By this method the number of positive cases has been more than tripled, and it is the only way in which the identification of the malaria species can be accurately done.—REGINALD D. MANWELL AND CARLTON HERMAN, *Department of Zoology, College of Liberal Arts, Syracuse University*. Supported by a grant in aid from the American Association for the Advancement of Science.

FISCHOEDERIUS ELONGATUS (POIRIER, 1883) AND *F. SIAMENSIS* STILES ET GOLDBERGER, 1910

A comparison of *Fischoeoderius elongatus* from Formosan *Bubalus bubalis* (Linné) and *F. siamensis* from *Bos taurus* slaughtered at the Kyoto abattoir has convinced me that the two species are unquestionably distinct, though they are regarded by Fukui (Jap. Jour. Zool., II (3), 318) as identical. In *F. elongatus* the body is usually more elongate and the eggs as fixed in alcohol and measured in water are 129-144 μ long by 72-84 μ broad, while in *F. siamensis* the body is rather plump and the eggs measure in the same condition 144-156 μ long by 87-96 μ broad.—SATYU YAMAGUTI, *Laboratory of Parasitology, Kyoto Imperial University, Kyoto, Japan*.

A METHOD FOR THE CULTIVATION UNDER STERILE CONDITIONS OF THE LARVAE OF *TAENIA CRASSICOLLIS*

Sarcoma of the liver of the rat has been induced by means of the *Taenia crassicollis*. The ova are obtained from the feces of infested cats and are fed to young rats. The embryonic membranes are digested by the intestinal juices of the rat which liberates the oncosphere and permits it to penetrate into the blood vessels of the portal circulation. In this way it reaches the sinusoids of the liver where it continues to develop within a cyst contributed by the host. After 8 to 22 months many of those cysts become transformed into sarcomata (Bullock, Curtis and Dunning, 1920, Proc. N. Y. Path. Soc. n. s. 20: 149 and 1933, Amer. J. Cancer, 17: 804).

The optimum time for removing the larvae from the liver of the rat is 15 days after ingestion of the ova. At this time the surface of the liver usually shows many pin-head size rudimentary cysts which can be aseptically and cleanly removed. These are placed in Locke solution and the young larvae are liberated by carefully puncturing the cyst with a sharp cornea knife. The larva can be identified macroscopically as a small blastocyst. The blastocyst and a few small fragments of the rudimentary cyst wall are transferred to a sterile test-tube and attached to the interior surface of the tube by means of a thin film of clotted chicken plasma. A fluid nutrient medium is added which is composed of 7 drops of balanced salt solution, 2 drops of chicken embryo extract and 3 drops of filtered horse serum. The test-tube is sealed with a rubber stopper and placed in the rotating carrier of an incubator at 37.5° C.

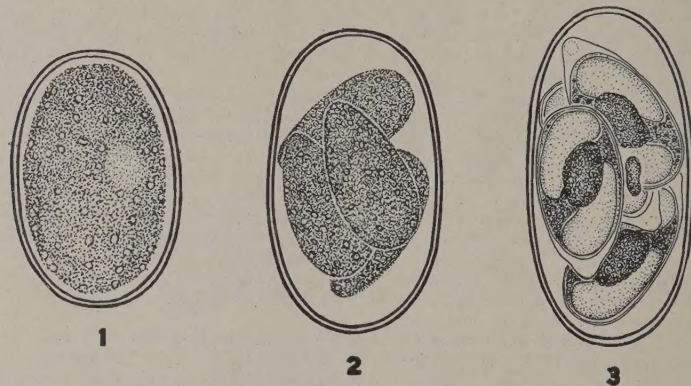
The details of this method are those of the Roller Tube technique of G. O. Gey which has been modified recently by W. H. Lewis. (1935 Contributions to Embryology). Although it is possible to cultivate the larvae alone, better results are obtained when small pieces of the rudimentary cyst wall are included. At the time of writing, a few larvae of *Taenia crassicollis* have been kept alive for 35 days. At this time the larva has proceeded to the invagination of the head with no evidence of sucker formation.—WM. MENDELSON, *From the Department of Surgery, Johns Hopkins University, and the Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.* Aided in part by a grant to Dr. W. H. Lewis from the International Cancer Research Foundation.

A COCCIDIUM FROM THE FOX SQUIRREL,
SCIURUS NIGER RUFIVENTER, GEOFFREY

Examination of fecal smears from a squirrel, *Sciurus niger rufiventer* (species identification through the courtesy of J. E. Guthrie), collected near Ames, Iowa, during September, 1934, revealed the presence of large numbers of oocysts of a coccidium belonging to the genus *Eimeria*. The squirrel had been placed for twenty-four hours in an electric refrigerator at a temperature of approximately 45 degrees F. before the intestinal and caecal contents were removed and mixed with a four per cent potassium dichromate solution. The material was kept in partially covered Petri dishes at a depth of approximately three-sixteenths of an inch. The content of the small intestines was kept separate from that of the caecum and large intestine. The mixture was stirred for the purpose of aeration at least every twelve hours during the sporulating period. Sporulation time was recorded from the time the oocysts were placed in potassium dichromate solution at room temperature.

The oocysts were, for the most part, restricted to the caecum and large intestine, although a few were found in the small intestine. The oocysts were almost cylindrical in shape with rounded ends. Measurements of 100 oocysts showed a length range of from 34.0 μ to 15.5 μ , and a breadth range of from 19.0 μ to 10.9 μ . The average size was 24.19 μ x 14.20 μ . Ratio of length to width varied from 1.35:1 to 2.15:1, the average being 1.70:1. The cyst walls were smooth and approximately uniform in thickness. The cytoplasmic mass did not completely fill the fresh oocyst. The oocysts had a characteristic pinkish to orange tint.

The micropyle was not distinct, although in some specimens the walls of the oocyst appeared to be slightly thinner at one end. After 21 hours in the potassium dichromate solution the cytoplasmic mass became rounded and situated in the center of the cell, *i.e.*, equidistant from the ends. Within thirty hours most of the oocysts showed cytoplasmic differentiation into four sporoblasts, and after thirty-three hours practically all showed sporoblastic formation and appeared as in figure 2. Completely sporulated oocysts were first noted after forty-eight hours



in the potassium dichromate, although the majority had not sporulated until the fifty-seventh hour. Within seventy hours sporulation was complete in practically all of the oocysts. A very small oocystic residual body was apparent. The sporocysts measured (average of twenty) $13.1 \mu \times 6.7 \mu$, and were more or less ellipsoidal in shape with a protruding nipple (figure 3). Two rather prominent sporozoites appeared in each sporocyst together with a large sporocystic residual body and what appeared to be additional residual material.

The question of the specificity of this coccidium is not an easy one to settle. Galli-Valerio (1922, Centralbl. Bakt., II Abt., 56: 344-347), Möller (1923, Inaug.-Dissert., cited by Yak. *et al.* 1931), and Yakimoff *et al.* (1931, Arch. f. Prot., 73: 487-490) have described similar species of *Eimeria* from other species of squirrels. *Eimeria sciurorum* Galli-Valerio (1922) from *Sciurus vulgaris* var. *alpina* measures $24 \mu \times 15 \mu$, is more cylindrical than ovoid in shape, and is said to show a small micropyle. Möller's species from *Neosclurus carolinensis*, according to Yakimoff, had elliptical, sometimes cylindrical, oocysts, measured 22μ to $28 \mu \times 14 \mu$ to 18μ , and clearly showed a micropyle from 4μ to 5μ across. Möller was able to cross infect "native German squirrels." Yakimoff's species had oval oocysts measuring 31.4μ by 21.4μ , and a micropyle 7.2μ across. It seems likely that the morphological characters of the forms seen by Galli-Valerio and Möller were identical, and that it might be tentatively assumed that both were *E. sciurorum*. Although Yakimoff concluded that the form described by him was likewise this species, it seems that its oocysts were far too large. The mean measurements of our species are very close to those submitted by Galli-Valerio and the shape corresponds closely. It may be assumed tentatively that the fox squirrel, *Sciurus niger rufiventris*, serves as the host for *Eimeria sciurorum*. Cross-infection experiments and further life-history studies would be necessary, however, in order to definitely prove this assumption. The fact that the micropyle showed only indistinctly in our species may indicate a specific difference, but since we have not observed the European species we cannot at present check this point.—EDWARD F. KNIPLING AND ELERY R. BECKER, Iowa State College.

SUMMER MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

At the Pittsburgh meeting of the American Society of Parasitologists the Council voted to refer the question of a summer meeting, in conjunction with the American Association for the Advancement of Science, to the local membership at Minneapolis. There was considerable question as to the advisability of attempting such a meeting and the responses to a questionnaire sent out indicated that there would be a very slight attendance of members of the Society. However, it was later decided to hold a session in conjunction with Section N, the medical section of the American Association.

Such a session was held on Friday morning, June 28th. There was presented a program of invited papers dealing largely with problems of entomology. This was highly appreciated by an audience of over one hundred.

Dr. R. R. Parker, of the United States Public Health Service, discussed the distribution and host relationships of various species of ticks of the United States which are known to be concerned in the transmission of human disease. Bringing as he did the latest and most authoritative data on this important topic, his presentation was very much appreciated.

Dr. R. G. Green, of the Department of Bacteriology, of the University of Minnesota, presented the results of his recent studies on the role of fleas and ticks in the spread of tularaemia in man and animals. The cottontail rabbit is highly susceptible to this disease and it appears that fleas are a factor in its maintenance in the late winter when ticks are rarely present. Tick infestation of rabbits reaches its maximum in August or September in Minnesota and is subject to marked variations from year to year.

Dr. Richard P. Strong, of Harvard University, discussed "Problems of Onchocerciasis in Africa." Numerous lantern slides were shown, illustrating the skin lesions and the eye symptoms produced by the worms. The conditions favoring development of the *Simulium* vector and the problem of animal reservoirs were considered.

Dr. R. A. Wardle, of the University of Manitoba, directed attention to the fact that recent reports on the distribution of the broad tapeworm, *Diphyllobothrium latum* in Canada might be based on incorrect determinations. His studies led him to believe that the *Diphyllobothrium* commonly found in dogs but very rarely in man in Manitoba was, in reality, *D. cordatum*.

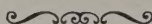
The demonstration program of the American Society of Parasitologists in the afternoon attracted much favorable attention. On the whole, the program of the day drew an unexpectedly large group and demonstrated that there is a widespread interest in problems of animal parasitology.—WM. A. RILEY.

CORRECTION

In the June issue of the JOURNAL (Vol. 21, No. 3) in the paper by Ackert, Porter and Beach there occurred a very unfortunate error on pages 208 and 209. In printing the paper the graphs were mixed. The graph on page 209 which is labelled Fig. 2 is really Fig. 1 and that on page 208 is Fig. 2. Fortunately this error was discovered in time so that it could be noted in the reprints.

Portraits of Parasitologists

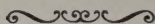
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